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STUDIES CONCERNING THE ALIMENTARY ABSORPTION OF WATER AND TISSUE HYDRATION IN RELATION TO DIURESIS.

By H. HELLER and F. H. SMIRK[1].

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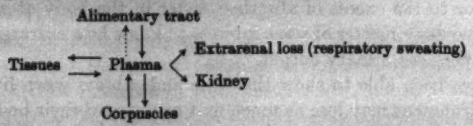
Our primary interest in this work has been the concurrent study of water diuresis and water storage in tissues both normally and under experimental conditions which inhibit the formation of urine, and it will be the object of these papers to show how numerous and interrelated are the factors which may modify or prevent the response of the kidneys to water administered by stomach tube.

To this end we have tried to determine how a dose of water received by the alimentary tract is normally distributed about the body and how the distribution is altered by measures which reduce the urinary output.

The disposal of this additional water throughout the animal body may be represented diagrammatically as follows:



For clarity we have subdivided our investigations of the above system.

Part I. The alimentary absorption of water in the rabbit, guinea-pig and rat takes place at different rates and, from this alone, differences in the responses of the kidney to water administration would be expected. Not only is this so but we find there is no parallelism between the load of absorbed but unexcreted water and the rate of urine formation at a given moment.

Although the times and rates of absorption and diuresis and the time relationships between them vary with the species of animal they appear to be fairly constant for any one species.

[1] Beit Memorial Research Fellow.

Our experiments have been made on the rabbit, rat and guinea-pig, and from indirect evidence which will be presented by one of us at a later date it seems that the absorption-diuresis relations in man bear most resemblance to those of the rat.

In the rat absorption from the alimentary canal may be studied readily by a statistical method; sets of rats being killed at varying times after water administration and the average amount of absorbed water determined by weighing the gut. In the guinea-pig this method of study is possible but less accurate because of errors introduced by the larger amount of residue in the lumen of the alimentary canal, and in the rabbit for this same reason it would be even harder to obtain reliable results.

In the rabbit therefore we have studied water absorption indirectly by determining the increased water content of blood, muscle and skin. The sources of error incidental to such a method have been controlled as far as possible.

Part II. The amounts of water lost extrarenally by small animals is large relative to their respective weights. Usually the loss takes place by the respiratory tract. A rat may lose as much as 1 p.c. of its body weight per hour and in a warm environment more is lost.

Now in Part I it is shown that the rate of urine formation is not parallel to the load of absorbed but unexcreted water in the body; nor is it parallel to the degree of plasma or whole blood dilution [Smirk, 1932]. Since diuresis is neither proportional to the degree of blood dilution nor to the excess of absorbed water in the body at any given time, it becomes a matter of some interest to know how extrarenal losses of water affect the urinary output.

We have been able to show that rats and rabbits when living in a warm environment may lose as much as 4 or 6 p.c. of their body weight in a relatively short time. So that if these animals are depleted of water and then are given 4 or 5 p.c. of their original body weight of water they may have, compared with their resting condition, only a slight excess of water in the body (as in the rabbits) or even a deficit of water (as in the rats). Nevertheless in both rats and rabbits the diuresis which follows this new supply of water is not very much less when the animal has been depleted of water previously, than in control experiments upon the same animals where there has been no preliminary water depletion and any additional water given constitutes an excess. Diuresis may apparently occur when there is no excess of water in the body. This leads us to a point of clinical interest.

The reduced urinary output of febrile patients is usually considered

to be the result of increased extrarenal loss of water. In rabbits and rats considerable extrarenal loss of water did not greatly reduce the urinary output and it appeared a suitable opportunity for testing whether an increase in temperature would produce oliguria in animals as in man and whether this reduction in urinary output might be in part due to some effect of pyrexia other than increased extrarenal loss.

The posture of an unanæsthetized animal makes a great difference to its powers of thermo-regulation and secondarily to its urinary output. This subject—of general importance in experimental work—is briefly dealt with.

Part III. In Part I it was found necessary to have a control observation upon the increase in the water content of muscle when water was absorbed normally but was not excreted by the kidneys. For this purpose use was made of the inhibitory action of pituitrin upon water diuresis. It was demonstrated that pituitrin did not cause any significant diminution in alimentary water absorption in the rat, and the presence in tissues of absorbed but unexcreted water was clearly demonstrated in the rabbit.

Although these results were originally obtained for another purpose they appear to provide additional evidence that the anti-diuretic action of the pituitary hormone is of primarily renal origin. The distribution of water in the blood tissues and serous cavities makes it unlikely that the pituitary hormone operates through any general change in the physico-chemical states of membranes or tissue proteins. For this reason the pituitary work has been treated separately.

Part IV. In clinical surgery and in experimental physiology it may be observed that the urinary output is changed by anæsthesia and laparotomy. This influence of anæsthetics upon diuresis is naturally of importance in the control of any renal experiments for which an anæsthetic is required, and the matter is interesting in relation to the nervous control of water metabolism.

From the school of Pick numerous papers [Kugel, 1929; Bonsmann, 1930 and Truemann, 1931, etc.] have been published which indicate that most anæsthetic substances inhibit a diuresis produced by the alimentary administration of water.

The influence of an animal's water absorption rate upon its urinary output (to be described in Part I) raises the question of whether anæsthetics may influence diuresis by delaying absorption or whether the anæsthetic acts upon an extra alimentary system, its effect being manifested after absorption has taken place. In regard to this last point Hicks and Smirk [1930] have demonstrated that when a blood dilution

has been produced by administration of water to rabbits the subsequent administration of chloretone causes a reconcentration of the blood and diminution of the urinary output.

Part I. The absorption and distribution of water during normal water diuresis in rabbits, rats and guinea-pigs.

Introduction.

The determination of the normal relationships between the absorption of water in the alimentary tract, its storage in blood and tissues and its subsequent excretion by the kidneys is made difficult by the probability that changes in all three factors may well occur as a result of the experimental procedures necessary in their investigation. Particularly is this so where general anæsthetics are used. Thus ether, chloralose, chloretone, chloralhydrate, morphia, isopral, veronal, luminal and trional have been recorded as inhibitors of water diuresis; urethane, paraldehyde and veronal have been stated to increase the urinary output. The extensive and in part contradictory literature is briefly summarized by Kugel [1929], and it would seem that although the results obtained depend on the animal used, the depth of narcosis and the length of the experimental period, there is no doubt that ordinary narcotic substances produce marked alterations in the amount of urine excreted and are clearly unsuitable for the investigation of its normal progress.

Decerebrate or decorticate dogs not under the influence of morphia and allowed to recover for one or more hours from the volatile anæsthetic have been used in investigations of this type by Fee [1929], Bayliss and Fee [1930].

They obtained a water diuresis which appeared to follow the normal course and investigated the changes in blood concentration. This method has the advantage that the effects of emotional disturbances and of muscular exertion on water metabolism are avoided. In water-diuresis tests on rabbits performed before and not less than 12 hours after the use of any general anæsthetic Godlowski [1930] finds an increased urinary output after decortication and after decerebration, either an increase or decrease depending, it appears, upon the level of section. We have preferred, however, to use local anæsthesia for the removal of our tissue samples because in the investigation of a process perhaps intimately bound up with the nervous system we hesitated to begin our investigations

by removing an important part of it. There remains with us the difficulty which Fee eliminated, namely, the effect of emotional disturbance in the animal on the course of diuresis. We have attempted to minimize this and to demonstrate by control observations in what degree and directions reliance may be placed on our results.

A. Rabbits.

Experimental procedures.

The removal and analysis of blood samples.

Blood samples were obtained from a marginal ear vein in rabbits, and heparin was our usual anti-coagulant *in vitro*. The blood dilution was investigated by several methods. The total solids of whole blood were determined by drying to constant weight at 107° C. The hæmoglobin was determined by conversion of a measured volume of blood to carboxy-hæmoglobin and colorimetric comparison of the samples taken before and after water administration. The plasma proteins were determined by Kjeldahl's method. The samples of blood removed were usually 0·5 c.c. The loss of blood during removal of muscle samples was slight. Probably the total loss before taking the second sample would not be more than 0·8 c.c. where muscle and blood samples alone were taken. The removal of 0·8 c.c. of blood does not appreciably affect the dilution of the second sample [Smirk, 1932].

The removal of muscle samples.

Two muscle samples were removed from symmetrical portions of the longissimus dorsi on either side, one before and one usually 2 hours after water administration. Local skin anæsthesia was maintained by 2 p.c. novocaine and 0·0004 p.c. adrenaline—the muscle not being infiltrated. The fibres of a suitable strip of muscle (1·5 × 0·5 cm.) are split longitudinally by a sharp scalpel, and the piece between may then be excised by short snips with a pair of scissors. The rabbit sits in a box and need not be held during the slight operation. The small wound is plugged with wool soaked in 2 p.c. novocaine to maintain anæsthesia and the animal is killed at the end of the experiment. The water content of the muscle is determined by drying to a constant weight at 107° C. The weight average of the muscle samples is about 0·8 g. A second muscle sample is obtained by the same procedure. In a few of the experiments performed in Vienna the wound was kept sterile, the animal preserved for a few days and an experiment repeated.

Control on the effect of local anæsthetics on a water diuresis.

Five rabbits received 2 c.c. of a solution containing 2 p.c. novocaine and 0·0004 p.c. adrenaline subcutaneously, 15 min. before the administration of 40 c.c. of water per kg. of body weight and then 4 c.c. of the novocaine-adrenaline solution 1½ hours after water administration. The amounts of urine formed in the 3 hours after water was given were 87, 77, 73, 71 c.c. (average 78·1).

In a set of experiments on the same rabbits where no local anæsthetic was given the corresponding outputs of urine were 95, 37, 102, 66, 75 c.c. (average 75·0).

Two rabbits received each 3 c.c. of 4 p.c. novocaine intraperitoneally and 1 hour later 72 c.c. of water by stomach tube. The amounts of urine over a period of 150 min. were 32 c.c. and 53 c.c. as compared with control diuresis tests in the same animals of 40 c.c. and 51 c.c.

In two other rabbits intraperitoneal injections of novocaine and adrenaline did not produce any significant change in urinary output.

There is no difference in urinary output which can be attributed to the use of local anæsthetics.

Control on the effect of the removal of blood samples from the ear veins, and of muscle samples from the longissimus dorsi on a water diuresis.

In a series of twenty-six experiments on thirteen different rabbits the diuresis following the administration of 40 c.c. of water per kg. body weight did not appear to be altered significantly by the procedures of removing blood and muscle samples, and in eight of these in which the

TABLE I.

	Control—urine samples only		Blood and muscle samples taken (animal unbound)	
Animal No.	Urine in 3 hours c.c.	Temperature change in °C.	Urine in 3 hours c.c.	Temperature change in °C.
17	60	−0·5	59	−0·2
16	64	−0·2	53	−0·0
15	43	−0·0	69	−0·1
10	51	−0·3	41	−0·1
11	20	−0·1	39	+0·5
12	34	−0·2	45	+0·4
13	29	−0·3	15	−0·1
14	30	−0·2	13	−0·0
18	35	—	29	—
19	67	—	121	—
20	5	—	53	—
21	22	—	20	—
22	20	—	21	—
Average	37		44	

rectal temperature was measured there was no change worthy of special note. The actual figures are given in Table I.

The average output of urine from the thirteen animals was 44 c.c. when muscle and blood samples were taken and 37 c.c. in the preliminary diuresis. It would therefore appear justifiable to consider that the uptake of water from the alimentary canal and the subsequent urinary output are not disturbed by taking samples of blood and muscle.

Control observations on the water content of muscle.

(1) Variations in the water content of the longissimus dorsi muscle from different batches of animals living under different conditions; and individual members of the same batch living under the same conditions (no additional water given).

TABLE II.

	Average water content p.c.	Water content of the muscle samples from individual rabbits p.c.
Batch I (5 rabbits)	74·8	75·0; 74·5; 74·5; 74·9; 74·9
Batch II (3 rabbits)	76·8	76·9; 75·8; 77·9
Batch III (2 rabbits)	—	75·8; 77·7
Batch IV (5 rabbits)	76·1	76·6; 76·1; 76·2; 76·1; 75·3
Batch V (2 rabbits)	—	77·7; 76·6
Batch VI (5 rabbits)	75·7	74·1; 75·8; 75·7; 76·3; 76·6
Batch VII (4 rabbits)	77·1	75·9; 78·2; 76·7; 77·7

It will be seen that as a rule the variations between batches of animals living under different conditions are rather greater than between members of the same batch. The members of the various batches of rabbits were not necessarily or usually of the same litter but were similarly housed and fed.

There did not appear to be any constant difference in the degree of muscle hydration between groups fed on oats and groups fed on cabbage.

(2) Variations in the water content of longissimus dorsi muscle taken from the same animals at an interval of 2 or 3 days (no additional water given).

It will be seen that at different times variations of muscle hydration are observed in animals which have at all times been allowed free access to food and water.

TABLE III.

		Percentage water content of the muscle samples from rabbit				
No. of animal ...		1	2	3	4	5
Batch I (5 animals)	1st sample	75·9	78·2	76·7	77·7	77·2
	2nd sample	75·9	78·3	76·6	79·3	77·1
		6	7	8	9	
Batch II (4 animals)	1st sample	76·6	76·1	76·2	76·1	
	2nd sample	75·0	74·5	74·5	74·9	
		10	11	12		
Batch III (3 animals)	1st sample	75·3	77·7	76·6		
	2nd sample	74·9	75·8	77·9		

(3) The constancy of longissimus dorsi muscle samples taken at nearly the same time from symmetrical parts on either side (no additional water given).

TABLE IV.

	Percentage water content of muscle samples from individual rabbits				
No. of animal ...	6	5	13	14	3
Sample from right side	76·6	77·1	77·8	76·9	76·6
Sample from left side	76·4	77·1	77·7	77·0	76·5

Symmetrical parts of the same muscles at the same time have equal water contents.

(4) Variations in the water content of muscle samples from symmetrical parts of the longissimus dorsi and vastus externus taken at intervals of 2 hours (no additional water).

TABLE V.

		Percentage water content of muscle samples from individual rabbits		
No. of animal		15	16	17
Longissimus dorsi	1st sample	77·4	77·2	76·4
	2nd sample	78·4	76·7	76·1
Vastus externus	1st sample	79·5	79·2	78·3
	2nd sample	79·3	77·8	77·4

As would be expected there is a tendency for the water content of muscle to be diminished slightly over a period of 2 hours owing to loss of water by the renal and respiratory systems. In all samples the water content of vastus externus has been greater than that of longissimus dorsi. The changes in water content are not equal.

(5) The detection of the additional water in muscles when its excretion by the kidneys is prevented: the instance chosen being the inhibition of diuresis by pituitary hormone.

It will be shown in Part III that although the uptake of water is not equal in all muscles yet there is no doubt that when water is absorbed but not excreted by the kidneys there is a substantial increase in the water content of muscle, often as much as 2 or 3 p.c.

The storage of water in muscle and blood during a normal water diuresis.

The following table gives the changes in blood concentration and in the water content of muscle samples taken before and after the administration of 40 c.c. of lukewarm tap water per kg. body weight to rabbits by means of a stomach tube. The resulting urinary outputs have been given in Table I, where they are compared with a previous set of experiments in which water was given but no blood or muscle samples were taken. It was shown that these additional procedures had no significant influence on the diuresis.

TABLE VI.

Animal No.	Blood concentration following water expressed as a percentage of the original concentration: Time after water administration 30 min.	90 min.	120 min.	Methods of determining the water content of blood	Water content of muscle in g. water per 100 g. muscle: Before water given	Time after water administration 90 min.	120 min.	Difference in water content p.c.
17	95·0	96·8	—		—	—	—	—
16	97·5	94·2	—		76·6	76·6	—	+0·0
15	96·0	91·4	—		77·7	78·1	—	+0·4
10	—	93·8	—	By percentage of solids in blood	76·6	76·6	—	+0·0
11	—	106·0	—		76·1	76·1	—	+0·0
12	—	96·3	—		76·2	76·6	—	+0·4
13	—	95·2	—		76·1	75·0	—	−0·1
14	—	104·0	—		75·3	75·7	—	+0·4
18	—	—	91·4	By dilution of proteins det. by Kjeldahl's method	74·1	—	74·2	+0·1
19	—	—	97·0		75·8	—	76·3	+0·5
20	—	—	—	—	76·3	—	76·7	+0·4
21	—	—	—	—	76·6	—	78·1	+1·5
22	—	—	—	—	75·7	—	76·4	+0·7
								Av. +0·33

It will be seen from Table VI that the degree of blood dilution varies considerably from one experiment to another, but the most general figure obtained is a reduction in the concentration of solids to 95 p.c. of their amount in the undiluted blood. Twice there was no dilution and the blood appeared to be more concentrated. Methodical error is unlikely and there are numerous difficulties attached to the interpretation of blood-dilution experiments such as the changes due to splenic contraction, changes in blood concentration following the muscular exercise of struggling or following alterations in blood-pressure.

In view of the possible difficulties introduced by slight losses of blood during the removal of muscle samples and the many conflicting statements in the literature concerning the presence or absence of a blood dilution in the course of water diuresis, a short separate study of this matter has been made by one of us [Smirk, 1932]. It was shown that in rabbits a whole blood dilution of 2 or 3 p.c. is usual, and that the larger part of this additional water is held by the plasma.

It will be observed from Table VI that the uptake of water in muscle is slight, and the total quantity of water represented by this in the animal's body is not great. The average change in the water content of the muscles is 0·33 p.c.

The average percentage weight of the striated muscle and of the skin in rabbits.

For further calculations it is of importance to know the relative amounts of muscle and skin in rabbits. In a series of nine animals the striated muscle accounted for approximately 40 p.c. and the skin with fur 12 p.c. of the total live weight. It is probably fair to assume another 5 or 8 p.c. for unstriated muscle, so that the weight of muscle in a 2000 g. rabbit will be about 900–950 g. Since most of our rabbits weigh 2000 g. and we may assume approximately 1000 g. of muscle with an average water percentage of 75 this mean change of 0·33 p.c. represents about 13 c.c. of additional water. In only two of the thirteen experiments was the water increase in muscle as much as 0·6 p.c. at a period of 1½–2 hours after water administration.

It must be remembered that if the above calculation is applied to individual muscle samples instead of to representative averages it will give only approximate indications of the water stored in muscle.

The storage of water in skin during a normal water diuresis.

In a short series of five animals small samples of skin (+ hair) were removed under local subcutaneous anæsthesia. The samples were cleaned of subcutaneous tissue and their water content was determined before, and 90 min. after, water administration by the method described for muscle.

The uptake of water was rather variable and appeared to be somewhat greater than that of muscle. The actual increases in water content were + 3·5, + 1·1, − 1·6, + 0·6, + 5·5 p.c. (average 1·8).

The necessity for local anæsthesia introduces a source of error.

During the normal diuresis in rabbits there does not appear to be any great increase in the water content of the muscles. Occasionally rabbits

are met where the normal output of urine in response to water administration is very low, yet the increase in the water content of muscles is as a rule no greater in these animals than in those where the output of urine is much greater (see Tables I and VI).

It will be shown later (Part III) that when diuresis is prevented or reduced by pituitary hormone a considerable increase in the water content of the muscle is obtained. It would appear from this that so long as the water given has been absorbed and is not yet excreted there will be a definite increase in the water content of muscle—say 2 or 3 p.c. The absence of such an increase in rabbits where the diuresis is poor is strong presumptive evidence that the water has not been absorbed. A possible alternative explanation would be that pituitrin might increase the absorption of water, and muscle hydration result as much from an increase in the uptake as from the diminished output. There is, however, no evidence in favour of this hypothesis, and in rats under the influence of pituitrin the water absorption curve is practically identical with the normal (see Part III).

The excess of water which is stored in muscle and blood prior to excretion is not often more than 20 c.c. when 80 c.c. have been given, and it appears in the rabbit that the excretion rate may lag only a little behind the alimentary absorption rate.

As the absorbed water is largely stored in the muscles when the normal renal response is inhibited by pituitrin it is of interest to know if the same relations between absorption and excretion hold in animals where water absorption takes place more rapidly. We therefore decided to study the diuresis in relation to the water absorption from the alimentary canal in rats and guinea-pigs.

B. Rats.

Since the rat is a smaller and less docile animal it would not be easy to obtain muscle samples and use their water content as an index of water storage in tissues. For the direct study of alimentary water absorption however the rat is much more suitable than the rabbit, and if in addition the urinary output is known we can deduce whether there is any appreciable storage of water in tissues as a normal phenomenon of water diuresis.

The method of study we have selected is as follows. A rat is weighed. 5 p.c. of its body weight of warm water is administered by a small stomach tube (No. 8 catheter). The animal is returned to its cage and at a selected

time after giving the water it is killed by a blow on the head and the alimentary canal is dissected out and weighed together with its contents. The longer the waiting period after water administration the more water is absorbed, and with a sufficiently large number of rats killed at varying times after giving water a normal average absorption curve may be constructed.

If now a series of similarly treated rats are also given 5 p.c. of their body weights of water, an average of their urinary outputs may be taken as a normal average curve of diuresis corresponding with the average absorption curve already obtained. The urine of individual rats can be collected by keeping the animals in small wire cages. On shaking the cage or taking hold of the animal's tail it voids urine even if only a little has been formed. The effectiveness of this method has been demonstrated by subsequent examination of the urinary bladder, so that by this means it is possible to obtain the precise onset of diuresis. The urine is collected on cotton-wool swabs and weighed.

Results.

The investigations on water absorption are summarized in Tables VII and VIII, from which may be constructed the two water-absorption curves of Fig. 1. The first gives the rate of water absorption in

TABLE VII. Animals allowed free access to water.

Time after giving water (min.)	Number of rats used	Weight of the alimentary canals and contents expressed as a percentage of body weight	Average
20	5	8·9; 8·6; 7·9; 7·8; 9·2	8·5
30	4	9·3; 8·2; 7·8; 8·0	7·7
	4	6·6; 7·1; 6·4; 7·9	
45	6	5·5; 6·5; 6·6; 6·5; 6·1; 5·9	6·2
60	5	6·6; 6·2; 7·1; 6·2; 6·2	6·5
75	7	4·6; 10·3; 6·4; 7·8; 5·9; 5·5; 5·0	6·5
120	6	6·8; 6·2; 7·7; 7·6; 5·9; 4·8	6·5
Controls, no extra water given	10	6·0; 5·9; 6·1; 5·0; 5·1; 5·1; 6·2; 5·7; 5·2; 5·0	5·5

TABLE VIII. Animals deprived of water overnight.

Time after giving water (min.)	Number of rats used	Weight of the alimentary canals and contents expressed as a percentage of body weight	Average
15	3	8·0; 8·4; 9·2	8·5
30	9	9·60; 6·5; 4·8; 6·9 (av. 6·9)	6·8
		6·4; 6·8; 7·7; 6·2; 6·5 (av. 6·7)	
45	7	6·9; 6·3; 6·4; 6·1; 6·1; 5·7; 5·7	6·2
Controls, no extra water given	7	5·3; 5·8; 6·7; 6·8; 7·0; 6·58; 6·20	6·3

animals allowed free access to water up to the time of the experiment, and the second gives the rate in animals deprived of water overnight. The percentage weight of the gut was 0·75 higher in the animals deprived of water (perhaps owing to their previous diet), and in order to render the two curves more easily comparable 0·75 was deducted from all the average values on Table VIII before graphing the results.

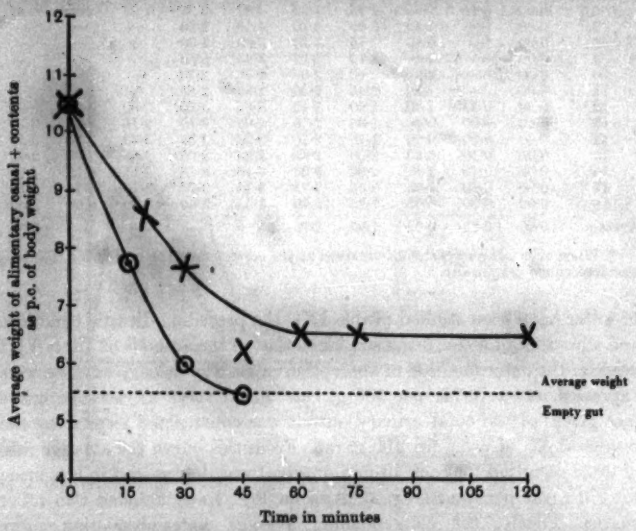


Fig. 1. Average water-absorption curves of rats: (*a*) previously allowed free access to water —×—×—, (*b*) previously deprived of water overnight —⊙—⊙—. The average weight of the empty gut is 5·5 p.c. of the body weight and 5 p.c. of the body weight of additional water was given, making 10·5 p.c. the starting-point of the absorption curves.

It would appear that water absorption proceeds more slowly in animals allowed free access to water up to the time of the experiment than in animals previously deprived of water overnight. We do not as yet regard this difference in absorption rate as sufficiently proved, but consider that, firmly established, it would be relevant to the problem of the physiological regulation of intestinal absorption.

In eighteen rats the diureses in response to 5 p.c. of their body weights

TABLE IX. Total output of urine expressed as c.c. per 100 g. rat.

No. of Animal	Time after water administration (min.)*									
	0	20	40	60	80	100	120	140	160	180
1	0·00	0·01	0·25	1·90	2·55	2·75	2·86	2·94	2·96	3·00
2	0·00	0·14	0·50	0·95	1·72	2·20	2·30	2·32	2·35	2·35
3	0·00	0·50	2·04	3·40	4·05	4·17	4·27	4·35	4·42	4·50
4	0·00	0·04	0·10	0·23	1·10	2·05	2·37	2·66	2·95	3·25
5	0·00	0·07	0·20	0·42	1·37	1·85	2·00	2·05	2·10	2·15
6	0·00	0·15	0·65	1·12	1·83	1·87	1·93	1·97	2·00	2·04
7	0·00	—	1·40	3·85	4·10	4·15	4·20	—	—	—
8	0·00	—	0·85	1·95	3·20	3·80	4·00	—	—	—
9	0·00	—	1·00	2·45	3·15	3·45	3·60	—	—	—
10	0·00	—	0·95	2·00	2·60	2·70	2·75	—	—	—
11	0·00	—	1·30	2·45	2·75	2·85	2·85	—	—	—
12	0·00	0·35	1·40	2·80	3·40	3·65	3·75	3·80	3·80	3·80
13	0·00	0·00	0·40	1·05	2·05	3·00	3·10	3·10	3·10	3·10
14	0·00	0·20	0·70	1·75	2·70	3·30	3·35	3·35	3·35	3·35
15	0·00	0·10	0·80	2·00	2·85	2·95	2·95	2·95	2·95	2·95
16	0·00	0·70	1·60	2·80	3·95	4·10	4·15	4·15	4·15	4·15
17	0·00	0·60	1·95	3·35	3·75	3·75	3·75	3·75	3·75	3·75
18	0·00	0·20	0·55	1·25	2·70	3·55	3·60	3·60	3·60	3·60
Average	0·00	0·23	0·93	1·95	2·75	3·10	—	—	—	3·35

* When urine samples were not obtained at the correct time the output for that time was determined graphically.

of water have been studied (Table IX), the preceding dietetic condition and allowance of water being similar to that of the animals of Table VIII used for the determination of absorption rates. The urinary outputs were expressed as c.c. urine per 100 g. rat. From these an average curve (not given) of the total urinary output was constructed expressing the results as c.c. of urine per 100 g. rat. From this curve the average rate of urine secretion for each 10-min. interval was determined in c.c. urine for 100 g. rat per hour (Fig. 2). Also on Fig. 2 are included two other curves. Firstly, the corresponding average water-absorption curve derived from Fig. 1, and secondly, a graph representing the average amount of absorbed but as yet unexcreted water in the body at any given time, which is obtained by subtracting the average total output of urine from the average volume of water absorbed at any given time and correcting for the average extrarenal loss of water (Part II).

It will be seen, on comparing the water absorption with the diuresis curve, that water absorption is well advanced before any notable increase takes place in the formation of urine. This is despite the fact that the dose of water is large: its equivalent would be 4 litres for an 80 kg. man but only 700 c.c. if the comparison were made by Rubner's surface formula. Also water absorption is practically complete some 15 or 20 min. before the height of diuresis is attained.

This lack of parallelism between water absorption and diuresis is further exemplified by the water-load curve. The average maximum load of absorbed but as yet unexcreted water is present in the rat 30 min. after giving the water. The rate of urine formation at this time is 1·2 c.c. per hour per 100 g. rat. It is another 30 min. before the kidney attains its maximum rate of urine formation.

Again, at the time when the rate of urine formation is greatest the load of absorbed water is 2 c.c. It also averages 2 c.c. ½ hr. after giving

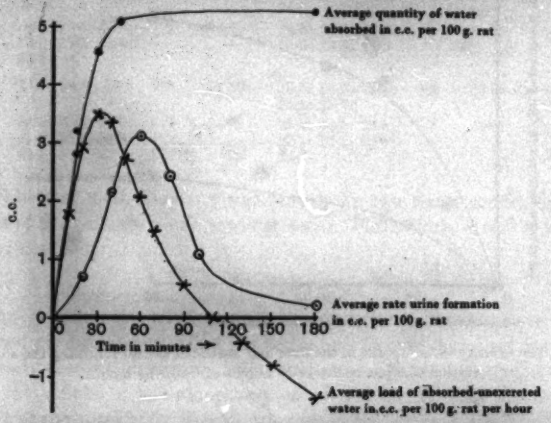


Fig. 2. The relationship between alimentary absorption, the water load and the rate of urine formation in rats. The water load is corrected for extrarenal losses of water from the respiratory tract.

water before absorption is complete, but in contrast at this time the rate of urine formation is only 0·3 c.c. per hour per 100 g. rat. With the same water-load distributed between the blood and tissues of the body the urinary outputs are regularly very different, being greater on the fall than on the rise of the water-load curve. Thus, when the water load is low shortly after giving the water and again as diuresis is subsiding, for equal water loads the urinary outputs are very different.

Since the uptake of water from the alimentary tract is clearly well in advance of diuresis it should be possible to demonstrate the increased water content of the body tissues which we have deduced, these increases being indicated approximately by the water-load curve. To obtain direct

confirmation of this samples of muscle and liver were removed from rats killed 20, 30 and 40 min. after the administration of water and, as controls, from rats in other ways similarly treated but to which no additional water had been given. A comparison of the water contents of the livers and muscles is given in Tables X and XI and in Fig. 3.

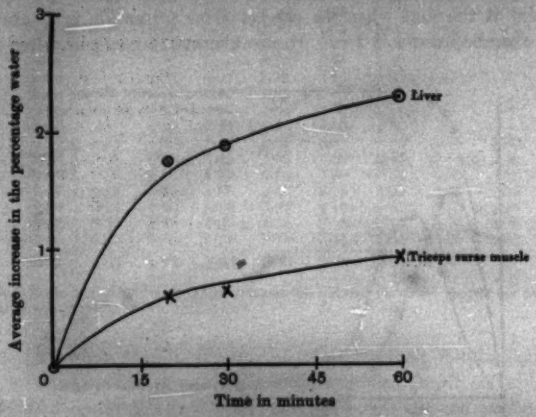


Fig. 3. The average water uptake in the liver and muscle of the rat following the administration of 5 p.c. of the body weight of water by mouth.

TABLE X. The water content of liver before and after administration of water. (Rats.)

Time after giving water (min.)	Controls (no water given) solids p.c.	No. of controls	Average of controls	Experiments (water given) solids p.c.	No. of exps.	Average of exps.	Difference in water content
20	28·4; 28·8; 28·8; 26·8; 29·3	5	28·44	26·4; 24·2; 26·6; 27·7; 28·2	5	26·6	+1·77
	28·0; 28·9; 28·9; 28·6	4	28·60	25·9; 25·8; 27·2; 27·2	4	26·52	+2·08
30	30·8; 32·2; 28·9; 30·6	4	30·62	29·8; 28·7; 29·6; 29·2	4	29·32	+1·3
	28·0; 28·9; 28·9; 28·6; 28·3; 28·9; 27·8; 28·1	8	28·44	25·9; 25·8; 27·2; 27·2; 26·9; 28·3	6	26·88	+1·56
60	28·3; 28·9; 27·8; 28·1	4	28·3	25·3; 25·5; 27·4; 24·9; 25·8	5	25·8	+2·5
	28·0; 28·9; 28·9; 28·6; 28·3; 28·9; 27·8; 28·1	8	28·44	27·4; 25·3; 25·5; 24·9; 25·8; 27·6; 27·7	7	26·31	+2·13

TABLE XI. The water content of muscle before and after administration of water. (Rats.)

Time after giving water (min.)	Controls (no water given) solids p.c.	No. of controls	Average of controls	Experiments (water given) solids p.c.	No. of exps.	Average of exps.	Difference in water content
20	23·4; 23·3; 24·0; 24·8; 24·1	5	23·9	23·8; 22·8; 24·1; 22·7; 23·1	5	23·3	+0·61
30	24·9; 25·4; 24·0; 24·3	4	24·65	24·3; 24·1; 23·2; 24·3	4	23·97	+0·68
	27·0; 27·6; 26·9; 26·7	4	27·05	26·8; 24·6; 26·9; 25·6	4	25·98	+1·07
	24·9; 25·4; 24·0; 24·3; 25·5; 24·8; 24·2	7	24·73	24·3; 24·1; 23·2; 24·3; 24·3; 24·8	6	24·17	+0·56
60	25·5; 24·8; 24·2	3	24·83	23·5; 24·0; 24·1; 24·1; 23·7	5	23·88	+0·95
	24·9; 25·4; 24·0; 24·3; 25·5; 24·8; 24·2	7	24·71	23·5; 24·0; 23·5; 24·1; 23·7; 23·5; 24·1	7	23·77	+0·94

It will be seen that there is a significantly greater water content in the tissues of the animals which received water. Particularly is this so in the case of the liver.

C. GUINEA-PIGS.

The experiments relating water absorption to diuresis have been repeated on guinea-pigs using the technique already described for rats. The study of water absorption is however more difficult in the guinea-pig, since the weight of the alimentary canal and its contents are usually 20 p.c. of the total body weight, whereas in the rat they are 5 or 6 p.c. The addition of 5 p.c. of the body weight of water will therefore make a smaller proportional difference to the gut weight in the guinea-pig, and its absorption is less easily followed by statistical methods.

TABLE XII.

Time after giving water (min.)	Number of guinea-pigs used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
15	6	26·7; 21·7; 26·4; 34·3; 26·0; 24·6	26·2
60	20	27·4; 18·8; 24·9; 18·7; 24·5; 24·0; 21·8; 18·4; 21·2; 24·7; 19·0; 20·8	22·0
		20·3; 14·9; 18·2; 16·6; 18·0; 15·3; 13·0; 19·9	17·2*
Controls, no extra water given	18	22·2; 18·8; 23·8; 14·9; 22·5; 24·7; 21·7; 26·2; 20·2; 19·2; 16·8; 21·0	21·0
		11·5; 18·5; 15·3; 14·0; 17·5; 20·0	16·1*

* These two groups of guinea-pigs were of a different breed and were differently fed and housed.

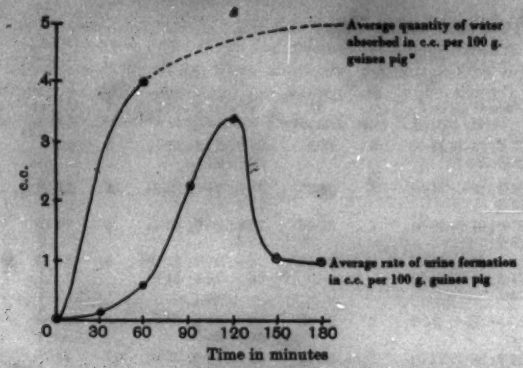


Fig. 4. The relationship between alimentary absorption and the rate of urine formation in guinea-pigs.

* The dotted line extension is presumptive, from analogy with the rat.

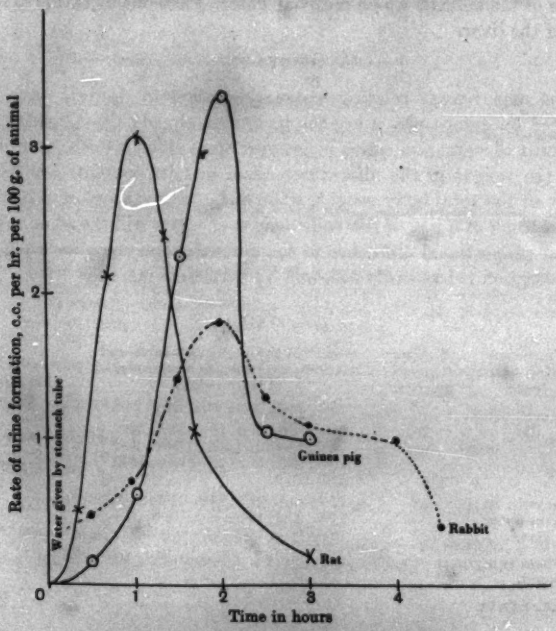


Fig. 5. The diuresis of the rat, guinea-pig and rabbit.

TABLE XIII.

Time after water administration (min.)	Total output of urine expressed as c.c. per 100 g. guinea-pig					
	Animal 1	2	3	4	5	Average
0	0·00	0·00	0·00	0·00	0·00	0·00
30	0·00	0·20	0·00	0·10	0·00	0·06
60	0·65	0·75	0·00	0·25	0·25	0·38
90	1·60	1·35	0·40	0·95	3·60	1·58
120	2·60	3·20	2·82	2·50	4·75	3·17
150	3·20	3·75	3·63	3·35	4·75	3·74
180	4·35	3·85	4·35	3·85	4·75	4·23
210	4·35	3·85	5·35	4·95	4·90	4·88

Table XII and Fig. 4 summarize the results for the absorption and Table XIII and Fig. 4 for the excretion of water. On Fig. 5 the rates of urine formation in the rat, rabbit and guinea-pig are compared.

DISCUSSION.

The object of this paper has been to study the normal relationship between alimentary absorption, tissue hydration and renal excretion of water in the rabbit, guinea-pig and rat. In the guinea-pig and rat the rate of water absorption was studied statistically by killing sets of animals at varying times after water administration. But in the rabbit, owing to great variability in the weight of the gut and its contents, this method was found to be unsuitable. In rabbits therefore the absorption of water was studied indirectly by observing changes in the water content of small muscle samples taken with the aid of local anæsthesia. Our controls confirm the observations of Tashiro [1926] and Baer [1927] that the water uptake of different muscles in the same animal may be unequal, so that from changes in the water content of any single muscle it is not possible to draw accurate quantitative conclusions as to the total increase in water content of the body musculature. In our experiments no appreciable increase in the water content of symmetrical parts of the longissimus dorsi muscles was observed during the progress of a normal water diuresis in rabbits. If, however, the diuresis is interrupted by pituitrin so that the absorbed water is no longer excreted an increase is regularly observed in the water content of this muscle, and if this degree of increase were representative of the entire musculature, a large proportion of the administered water would be accounted for. So we may conclude that by our method it is possible to detect any appreciable storage of water in muscle.

Since there is no regular appreciable increase in the water content of rabbit muscle during a normal water diuresis it is very probable indeed

that no great storage of water takes place unless the excretion of urine is lessened (as by pituitrin). The average increase of muscle water of twelve animals was 0·33 p.c., and this in a 2 kg. animal with, say, 950 g. of muscle would represent less than 13 c.c. of additional water.

The results obtained on skin samples are more variable, but they are liable to greater errors. Sometimes larger increases in their water content are observed than have been described for the samples of muscle, but since the average weight of the skin in nine rabbits amounts to only 12 p.c. of body weight these increases do not represent more than a few c.c. of additional water.

In guinea-pigs and in rats it is clear, however, that water absorption is well in advance of diuresis. The direct study of water absorption from the gut is more difficult and less accurate in guinea-pigs than in rats, since the gut forms as much as 20 p.c. of the total body weight in the former and 5 or 6 p.c. in the latter animal. When our results on guinea-pigs are treated statistically it is probable, however, that most (4/5ths) of a 5 p.c. body weight dose of water is absorbed in 60 min. and practically certain that the maximum load of absorbed but unexcreted water occurs some 20–40 min. before the height of the diuresis. In rats this is still more clearly demonstrated because of the greater certainty of our results on water absorption.

There is no doubt that in rats, and it is highly probable in guinea-pigs, that the maximum load of water in the blood and tissues precedes the period of maximum diuresis, and that there may be no increase in urinary output at a time when a quantity of water equivalent to 3 litres in man has been absorbed. A statistical survey of the water content of muscle and liver from rats which had and rats which had not received water, demonstrates clearly the additional water content of these tissues at a time when there is no appreciable diuresis (see Tables X and XI, and Fig. 3). The water increase in liver is rather greater than in muscle, perhaps due to the greater water content of portal blood during absorption. The delay in the onset of diuresis has been often attributed to the fact that the portal blood must first pass through the liver. Although the liver takes up proportionally more water than muscle it is certain from our results that in the rat the total amount of water held in the liver is insufficient to cause any appreciable delay in diuresis. Thus when 5 p.c. body weight of water is given the amount of water held in the liver is less than 0·5 p.c. body weight even with abnormally large livers. This does not mean that the liver cannot be responsible for the delay in diuresis. Indeed Mollitor and Pick report a shortening of this delay

in dogs with an Eck fistula. It would appear, however, that it cannot be due to delay in the entrance of the water into the general circulation as a result of its storage in the liver, since the degree of storage is insufficient.

It would appear to be demonstrated quite clearly that the rate of urine formation at any moment is not proportional to the amount of extra water then present in the blood and tissues. There is a time lag of 20 or more min. between the maximum water load and the maximum diuresis.

In Fig. 2 it will also be observed that in rats the water-load curve falls below the base line. The urinary excretion and extrarenal excretion of water is therefore carried beyond the need of the animal to dispose of its excess. The total urinary output after giving 5 p.c. body weight of water averaged only 3·35 p.c. body weight in 3 hours and the non-fæcal extrarenal water loss was 3·69 p.c. body weight in the same time. The large extrarenal water loss was the main factor in carrying the water-load curve below the base line, since at this time the urinary output had lessened. Nevertheless it will be shown in Part II that the fall of the water-load curve does not determine this difference in magnitude between the dose of water given and the output of urine.

Observations made by Priestley upon human subjects have indicated that following the administration of large doses of water the general blood dilution, as estimated by hæmoglobin and the dried weight of blood, is slight, but that a dilution of electrolytes is readily demonstrated. Priestley attributes these changes to the withdrawal of salts from the blood: a natural consequence of the storage of water in tissues. In the rat this storage of water is demonstrated both by direct analysis of tissues and by the simultaneous study of water absorption, urinary output and the loss of water extrarenally. In the guinea-pig water storage also takes place.

In the rabbit, however, it has been shown in this paper that there is comparatively little water storage and that water excretion appears to lag only a little behind water absorption. One would, therefore, anticipate that in the rabbit the reduction in the electrolytes of the blood would not be so marked. This has been shown to be the case by one of us [Smirk, 1932].

Further evidence upon this is offered in a subsequent paper of this series. Verney explains the delay in the onset of diuresis by the time required for the disappearance of the pituitary hormone from the blood. Such a delay might arise in this way or from the time required to mobilize

some diuretic substance from the tissues. It is not likely that a delay of as much as 20–30 min. would be observed if water diuresis were the result of a direct action of the nervous system upon the kidney.

Conclusions.

1. In the guinea-pig and in the rat alimentary absorption is well in advance of diuresis. In rats, given 5 p.c. of their body weight of water, a weight equivalent to 3 litres of water for a man is usually absorbed at a time when no increase in urinary output has taken place.

2. The rate of uptake of water from the gut of a rabbit is probably not much more rapid than the rate of excretion. There are, however, difficulties in the quantitative interpretation of results upon which this observation is based.

3. The rate of urine formation is not proportional to the then existing degree of hydration in muscle, liver and blood.

We desire to thank Profs. T. R. Elliott and E. P. Pick for their helpful criticism and advice, and Dr Priestley for his kindness in affording us an opportunity for discussion.

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Part II. The influence of binding an animal, of its body temperature, and of extrarenal losses of water upon diuresis.

Introduction.

The influence of the binding of a rabbit upon its urinary output is intimately related to changes in body temperature and extrarenal losses of water. It would be difficult to consider these subjects separately.

Thus when an unanæsthetized rabbit is bound lightly on its back its temperature falls. If it is bound and kept in a room at body temperature then its temperature rises; and the increase in temperature of bound rabbits in a room at 37° C. is greater than the relatively slight increase in temperature of the unbound animals.

In preliminary experiments we found that bound rabbits kept in rooms at ordinary temperature had a normal urinary output: but in rooms at 37° C. a marked inhibition of urinary output. Unbound animals in rooms at 37° C. had slighter urinary inhibition. The degree to which such reduced outputs of urine could be attributed to increased extrarenal losses of water was next investigated and an attempt made to separate experimentally the effects of temperature and of extrarenal loss upon diuresis.

The effect of lightly binding an unanæsthetized rabbit upon its temperature and diuresis after water.

It has been stated by Molitor [1926] that as a result of the fright induced in an unanæsthetized rabbit by the simple act of binding it to an operating table an inhibition of diuresis results, the mechanism of which is a central nervous system reflex independent of the posture in which the animal is held. On the other hand, it is also well known that an increase in the urinary output may co-exist with emotional over-anxiety in man.

In our preliminary controls however we did not observe any reduction in the urinary output of bound animals. But since any such alterations might prove a fruitful source of misinterpretation it was thought advisable to pursue the matter further.

Results.

In a series of ten rabbits the diuresis which resulted from a given dose of water was compared with the diuresis from an equal dose of water subsequently administered to the same animals under similar conditions,

adding the one additional procedure of lightly binding the animal on its back to a small operating table. It was found that the average urinary outputs of bound and unbound animals did not differ appreciably (Table I), but that the rectal temperature of the bound animals had fallen—the average fall being 1·7° C.

TABLE I. The influence of lightly binding an animal upon its body temperature and upon diuresis.

	Unbound		Bound and allowed to cool	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.
1	106	—	88	−1·6
2	57	—	86	−2·6
3	87	—	89	−1·9
4	75	0·0	105	−1·1
5	69	−0·2	80	−1·5
6	67	−0·1	64	−1·9
7	106	−0·3	79	−1·5
8	126	—	135	—
9	109	—	57	—
10	42	—	57	—
Average	84·4	−0·2	84·0	−1·7

These experiments differ only from those described by Molitor in that we allowed the body temperature of the bound animals to fall whereas Molitor took steps to prevent cooling.

It would appear that the simple act of binding together with the resulting fall of body temperature does not produce any significant change in water output.

An inhibition of diuresis co-exists with a rise in body temperature.

Molitor prevented his bound animals from cooling by the use of heated tables, electric lamps or warm air chambers at 37° C., but he does not quote the body temperatures produced by these measures.

The temperature of our rabbits in a chamber at 37° C. rose, and the diuresis after giving water was considerably reduced not only in the bound animals but also in the unbound controls. The temperature increase was greater in the bound animals as was also the reduction in urinary output.

The average urinary output of the seven bound animals kept in a warm chamber was 16 c.c. and their average temperature increase 2·4° C., whereas under normal conditions their output was 81 c.c. and they had an average fall in temperature of 0·2° C. (Table III).

The average output of the five unbound animals in the warm air chamber was 37·5 c.c. and their average temperature increase was only 0·3° C. Under normal conditions these same rabbits had an output of 75·5 c.c. and a fall in temperature of 0·2° C. (Table II).

TABLE II. The effect of a warm environment upon an unbound animal.

	Unbound and not warmed		Unbound and warmed	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.
1	106	Average of 12 other animals −0·2	42	+0·2
2	57		30	+0·0
3	87	—	39	+0·6
8	61	−0·5	39	+0·0
9	66	−0·2	38	+0·8
Average	75·5	−0·2	37·5	+0·3

TABLE III The effect of a warm environment upon an animal gently bound on its back.

	Unbound and not warmed		Bound and warmed	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.
1	106	Average of 12 other animals −0·2	29	+1·8
2	57		16	+2·8
3	87		11	+2·4
4	75		24	+3·0
5	69		18	+3·7
6	67	−0·1	6	+2·1
7	106	−0·3	9	+1·2
Average	81	−0·2	16	+2·4

It would therefore appear that a rise in the temperature of a rabbit produced by keeping the surrounding air at body temperature is regularly associated with a very considerable lessening of diuresis.

Does the simple act of binding cause an inhibition of diuresis if the fall of body temperature produced by binding is prevented?

On a series of eleven rabbits the diuresis produced by 75 c.c. of water was compared in the same animals when bound and unbound. The rectal temperature was measured frequently during the experiment and in bound animals the temperature was maintained, where possible a fraction of a degree below the initial temperature, by transferring the animal to a warm chamber whenever the body temperature showed a tendency to fall.

In some animals the temperature fell more than was desirable. Group I, Table IV, comprises the animals where the temperature has not fallen more than 0·5° C. below the temperature in the control experiment, and Group II, Table V, comprises those animals where the temperature has fallen by more than this amount although as a rule less than in the animals of Table I where no attempt was made to prevent cooling.

TABLES IV and V. The effect of temperature changes upon the diuresis of rabbits.

TABLE IV. Group I.

	Normal		Bound and temperature maintained normal	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.
10	51	−0·3	15	−0·7
12	34	−0·2	50	−0·6
14	30	−0·2	17	−0·7
17	60	−0·5	20	−1·0
18	59 (cabbage)	−0·2	69	−0·4
19	101	−0·4	80	−0·6
20	35	−0·3	41	−0·5
Average	53	−0·3	42	−0·6

TABLE V. Group II.

	Normal		Bound and temperature slightly reduced	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.	Diuresis c.c. in 4 hr.	Maximum temperature change in °C.
11	20	−0·1	93	−0·9
13	29	−0·3	67	−1·7
15	43	−0·0	37	−1·4
16	64	−0·2	59	−1·9
Average	39	−0·15	64	−1·5

It will be seen from Table IV that the total urine formed by the seven animals of Group I was 42 c.c. in 4 hours when bound and the temperature was kept normal as against 53 c.c. when they were unbound. In Group II, which contains four animals, the fall in temperature averaged 1·5° C., and the total output when the animals were bound amounted to 64 c.c. as against 39 c.c. when they were unbound.

It would appear therefore that the act of binding may have some influence in inhibiting diuresis, since in experiments performed on the same series of animals maintained at the same level of body temperature and subjected to the same manipulations of bladder expression and measurements of rectal temperature, the additional procedure of binding

seemingly reduced the urinary output in four out of seven animals, the reduction in urinary output being 31 p.c.

It must be remembered, however, that when bound the animals had their rectal temperatures measured more frequently, and were being moved between a warm and perhaps stuffy chamber and a cool room according to the level of their rectal temperatures.

For the experiments described in Tables IV and V only animals were used with a fairly constant urinary output in successive diuresis tests.

It is clearly very difficult to separate the effect of binding from the changes in body temperature even when these are slight. It is possible that the direction in which the mechanisms of heat regulation are acting (hot → cold or cold → hot) play a part before any significant temperature change is observed.

The relationship of extrarenal loss of water to diuresis in rabbits.

A series of five rabbits previously fed on cabbage and oats and allowed an unlimited supply of water were deprived of food and water overnight. The diuresis and non-fæcal extrarenal losses were then determined over a period of 4 hours, after giving 4 p.c. of their body weights of warm water by stomach tube. The results of this experiment, conducted at ordinary room temperature (17° C.), were compared with the results obtained when a similar experiment was carried out in a room warmed to 35–36° C. In all experiments the animals were unbound.

It will be clear from Table VI that the sum of the average urinary and extrarenal losses of water is approximately equal in the two series

TABLE VI. The relation between renal and extrarenal water losses at different temperatures.

	Control observation at 17° C.		Experiment at 35–36° C.		
Experiment No.	Extrarenal loss g. in 3 hr.	Diuresis c.c. in 3 hr.	Extrarenal loss g. in 3 hr.	Diuresis c.c. in 3 hr.	Maximum temperature increase in °C.
1	13·5	30·5	51·0	26·0	+2·4
2	15·0	93·0	30·0	74·0	+0·9
3	10·0	93·0	41·5	38·5	+1·7
4	24·0	56·0	48·0	24·0	+1·6
5	24·5	46·5	88·5	22·5	+1·6
Average	17·4	63·8	51·8	37·0	+1·6

of results, but whereas in the experiments conducted at laboratory temperature the urinary output of water exceeds the extrarenal loss, the reverse is the case in the warm chamber. There is in fact a clear inhibition

of diuresis which would appear to be sufficiently accounted for by the increase in extrarenal loss of water.

It will be observed, however, that in experiments conducted at 35–36° C. there is a distinct increase of body temperature, and although

TABLE VII. The effect of previous water depletion upon a water diuresis.

	Controls (no previous water depletion)			Experiments			
Rabbit No.	Water given by stomach tube = 4 p.c. body weight c.c.	Amount of water in excess of normal c.c.	Diuresis during the subsequent 4 hr. c.c.	Amount of previous water depletion c.c.	Water given by stomach tube c.c.	Amount of water in excess of normal c.c.	Diuresis during the subsequent 4 hr. c.c.
22	88	88	78	70	88	18	61
23	91	91	86	85	91	6	43
24	81	81	71	67	81	14	65
25	90	90	38	67	90	23	99
26	86	86	17	59	86	27	71
27	123	123	129	44	123	79	107
28	112	112	91	57	112	55	64
29	114	114	62	49	114	65	70
30	105	105	127	43	105	62	86
31	114	114	114	46	114	68	60
Average	100·4	100·4	81·3	58·7	100·4	41·7	72·6

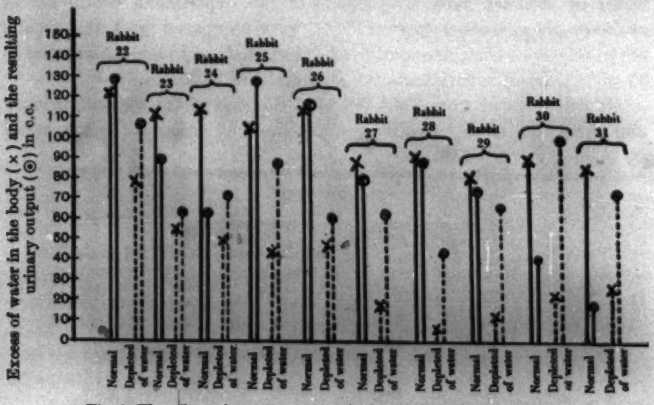

Fig. 1. The effect of previous water depletion upon a water diuresis.

extrarenal losses of water are without doubt increased it does not follow that the inhibition of urinary output is a result of this loss. It was decided therefore to produce a corresponding degree of extrarenal water

loss in a series of rabbits by keeping them in a warm chamber at 36° C., allow them to cool to normal body temperature and then to administer water. In this way it should be possible to separate the effects of extrarenal loss from those due to the slight increase of temperature.

It will be seen from Table VII and from Fig. 1 that although a previous water depletion usually diminishes the diuresis from a subsequent dose of water, the degree of diminution when present does not correspond to the amount of water lost and is always less than this.

The amount of water given by stomach tube was the same both in the experiments and in the controls, and averaged 100·4 c.c. in each. In the controls where there was no water depletion this amount given constitutes an excess of water above the initial quantity present, but in the experiments, owing to the previous water depletion, the excess of water only averages 41·7 c.c., although the same quantity of extra water has been administered per os. The diuresis which results averages in the controls 81·3 and 72·6 c.c. in the experiments. A diminution of 58·7 c.c. in the excess of water produced by the preceding water depletion causes a fall of only 8·7 c.c. in the urinary output. In the controls the urinary output over a period of 4 hours is usually rather less than the excess of water resulting from water administration, but in the experiments the urinary output is in all cases greater than the excess of water, and is usually considerably greater.

The changes described occur so regularly in each pair of observations that it is quite fair to consider these average figures as representative.

In contrast, the urinary output obtained when the animals were in the warm chamber was less than the diuresis in the experiments which we have just described, in spite of the fact that the extrarenal loss of water had been much greater in the experiments at room temperature.

It seems clear therefore that the inhibition of diuresis in a warm environment is not mainly the result of the increased extrarenal loss of water induced.

The relationship of extrarenal loss of water to diuresis in rats.

Since it is difficult to determine the rate of water absorption from the gut of a rabbit it was thought desirable to repeat our observations on rats and to ascertain whether the diminished urinary output resulted from interference with water absorption. It is also of interest to study the relationship of temperature, extrarenal loss and diuresis in a small animal where one would expect the part played by extrarenal loss to be greater.

The water-absorption rate of rats in a room at 37° C.

A series of five rats were fed for 3 days on bread and milk. They were allowed water up to within 1 hour of the experiment, but were deprived of food for 12 hours except that by mistake they had access to dry biscuit for a short time several hours before the experiment—there was no appreciable residue in the alimentary canal at the conclusion of the experiment.

The rats were placed in the warm chamber for 1 hour and then given 5 p.c. of their body weight of warm water and returned to the chamber. At the end of 40 min. they were killed and the alimentary canals removed and weighed together with their contents and expressed as a percentage of the total body weight.

The results obtained were 8·1, 6·6, 7·7, 6·7, 6·1 p.c., averaging 7·0 p.c. From similar experiments conducted at room temperature and described in Part I [Heller and Smirk, 1932] we may conclude from the average water-absorption curve obtained (Part I, Fig. 1) that the normal average is 6·8 for animals allowed water up to the time of the experiment. It is therefore clear that there has been no appreciable delay in water absorption. Any clear inhibition of urinary output in similarly treated animals is not to be attributed to diminished alimentary absorption.

The influence of a warm environment on renal and extrarenal water elimination.

A series of eleven rats, fed on bread and milk to within 12 hours of the experiment, were weighed and placed in a large incubator at 37° C. for 1 hour. They were then re-weighed, adding to their weights the weight of any fæces passed during this hour. Their urine was collected on cotton-wool swabs and was also weighed. 5 p.c. of their original body weights of warm water was next administered and the animals returned to the incubator. The resulting urine was collected at frequent intervals and the non-fæcal extrarenal loss was again ascertained by weighing. The urinary outputs and extrarenal losses are expressed as a percentage of the animal's total body weight and are compared with the results of similar experiments upon the same animals conducted in a normal environment at ordinary room temperature.

Table VIII compares the total outputs of urine and the extrarenal losses of these animals at laboratory temperature and at 37° C. Fig. 2 gives representative diuresis curves for the animals in the different temperature environments.

TABLE VIII. The relationship between diuresis and extrarenal loss in experiments on rats. Conducted at 37° C.

Rat No.	Urinary output per 3 hr. as percentage of body weight	Extrarenal loss per 3 hr. as percentage of body weight
24	1·19	4·15
25	1·54	4·60
5	0·66	5·40
7	3·25	4·85
8	4·00	3·70
9	3·65	3·35
12	3·26	5·85
13	0·98	10·40
14	1·36	8·10
15	1·40	4·35
16	2·05	7·40
Average	2·12	5·67

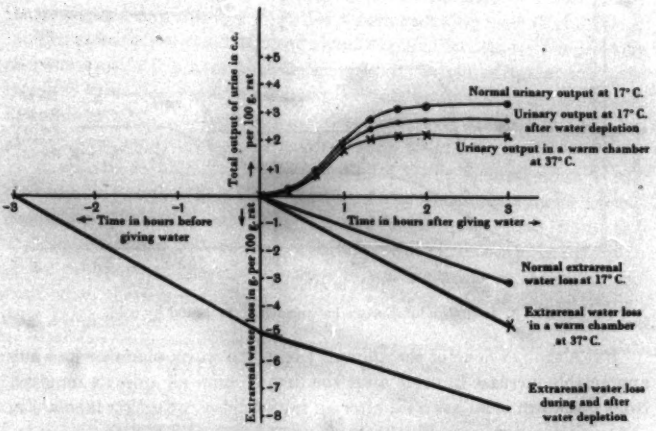

Fig. 2. The relations between environmental temperature, extrarenal water losses and diuresis.

It will be observed that there is a moderate diminution in the total urinary output and an increase in the extrarenal water elimination, the results being similar to those obtained in rabbits except for a lesser degree of renal inhibition.

Three more rats were only placed in the incubator after giving water. Their urinary outputs were 2·34, 2·92, 1·46 c.c. per 100 g. rat, and the extrarenal losses of water 6·0, 5·9 and 5·4 g. per 100 g. rat for the 3 hours.

The inhibition of diuresis is not well marked, and it appears necessary to have the animal in the warm environment for a short time before giving the water.

In five of the experiments from Table VIII the rats were removed from the warm chamber at the end of 2 hours. At this time it appeared that the secretion of urine had ceased, and from the previous experiments it seems justifiable to conclude that if they had been left in the warm chamber for the full 3 hours there would have been no further increase in urine formation during the last hour.

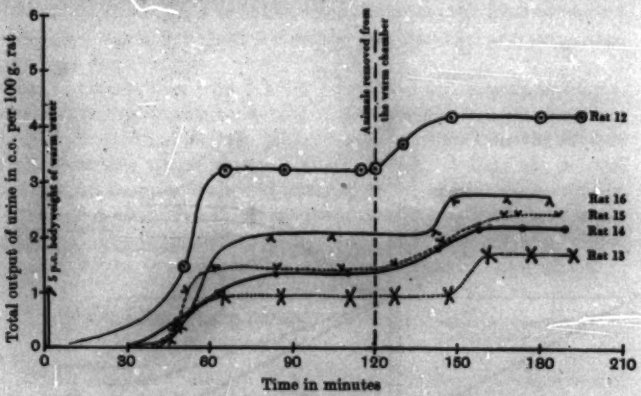

Fig. 3. Inhibition of diuresis by warmth and renewal by cold.

But although none of the animals left in the warm chamber had any appreciable increase in urine after the first 75 min. all animals removed from the warm chamber had, after an initial delay, a further increase in the urinary output (see Fig. 3).

This observation provides strong evidence that extrarenal loss of water does not sufficiently explain the urinary inhibition observed in the warm environment. If the sole explanation of the lessened urinary output was the increased extrarenal water elimination, then from the time the extrarenal water loss had been sufficiently great to inhibit urine formation one would expect no further increase in the urine secreted until the animal was given a fresh supply of water. This, however, is clearly not the case, and one must postulate some factor other than the water content of the tissues.

It is possible that a slight increase in body temperature may be present in rats as in rabbits, and may be the primary or a secondary cause of the reduced urinary output.

Water diuresis without water excess.

It is natural to suppose that water given to an animal depleted of water would first be used to make good the deficit, and that only the unneeded excess would be excreted. In the rat, as in the rabbit, it can be shown that this is not the case with moderate losses of water.

A series of eleven rats were placed in the warm chamber at 37° C. (with short intervals at room temperature) for a period of 3 hours and the non-fæcal loss of weight recorded. In most instances the loss amounted to more than 5 p.c. of the animals' weight, the normal loss at laboratory temperature being about 3·7 p.c. for a corresponding time (Table X).

The animals were then allowed about 1 hour at laboratory temperature and were given 5 p.c. of their original body weight of warm water by stomach tube. The resulting urinary outputs and extrarenal losses were determined in the manner already described, and the results again expressed as a percentage of the original body weight.

It will be seen in Table IX and Fig. 4 that the total urinary outputs are only slightly less than the outputs of the animals under normal conditions (Table X and Fig. 4), and it is quite clear from this that water diuresis follows approximately its normal course in the rat as in the rabbit when there is no excess of water present in the body, and even when there is actually a deficit of water in spite of the additional fluid administered.

TABLE IX. Diuresis without water excess in rats.

Rat No.	Extrarenal loss of water 3 hr. previous to giving water by stomach tube as p.c. of body weight	Amount of water given per os as p.c. of body weight	Residual water deficit	Urinary output in the 3 hr. after giving water as p.c. of body weight	Extrarenal loss in the 3 hr. after giving water as p.c. of body weight
3	4·1	5	−0·9	3·40	2·50
4	5·1	5	0·1	1·82	2·85
5	4·1	5	−0·9	1·60	3·20
6	5·5	5	0·5	2·67	—
22	6·0	5	1·0	2·87	—
23	6·6	5	1·6	3·05	—
7	6·0	5	1·0	3·10	2·80
8	4·3	5	−0·7	3·85	3·75
9	3·9	5	−1·1	3·75	3·75
10	4·2	5	−0·8	1·71	6·30
11	5·1	5	0·1	2·52	3·3
Average	5·0	5	0·0	2·76	3·56

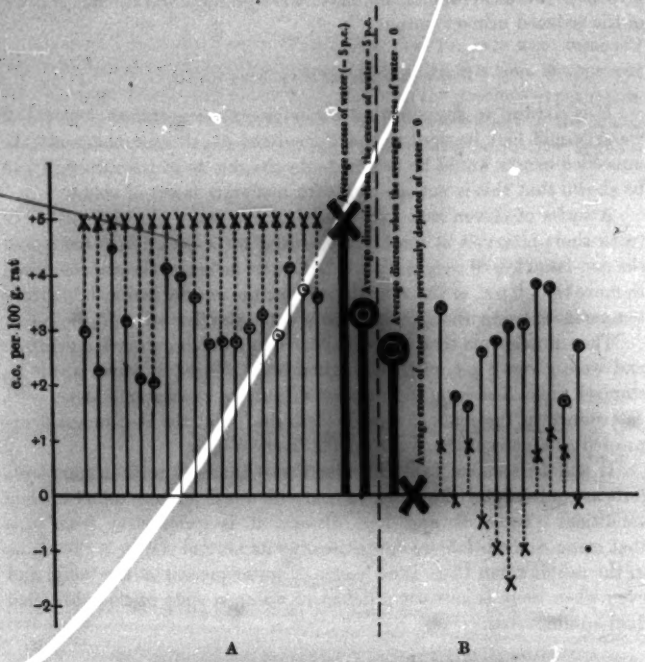

Fig. 4. Water diuresis without excessive water in the body.

Each vertical line represents one experiment. The height of the × above or below the zero line represents the excess or deficit of water in the body after the whole of the 5 p.c. body weight of water given has been absorbed, deficits being the result of previous water depletion. The height of the ⊙ above the zero line represents the urinary outputs resulting from water administration. The thick lines in the middle of the diagram represent the average values of the two groups of results.

A. Controls. (5 p.c. of body weight of water by stomach tube.)

B. Experiments. (5 p.c. of body weight of water by stomach tube, the rats being previously depleted of water [average depletion = 5 p.c.].)

TABLE X. Diuresis with water excess in rats (no previous water depletion).

Rat No.	Amount of water given per os as percentage of body weight = Excess of water in the body	Urinary output in the 3 hr. after giving water as percentage of body weight	Extrarenal loss in the 3 hr. after giving water as percentage of body weight
1	5	3·00	2·00
2	5	2·35	2·90
3	5	4·50	1·65
4	5	3·25	1·80
5	5	2·15	2·90
6	5	2·05	2·75
7	5	4·15	5·20
8	5	4·00	6·70
9	5	3·60	5·60
10	5	2·75	7·20
11	5	2·80	8·40
12	5	2·80	3·50
13	5	3·05	1·95
14	5	3·30	3·55
15	5	2·90	2·10
16	5	4·15	3·20
17	5	3·75	2·65
18	5	3·60	2·45
Average	5	3·29	3·69

It is therefore a reasonable deduction that the inhibitions of diuresis which were observed in these animals in the warm chamber could not be due to the relatively slight extrarenal losses, and in all probability were the result of the slight increases in body temperature which were observed in unbound rabbits under similar conditions (see Part I of this paper).

DISCUSSION.

In small animals such as the rat and rabbit the extrarenal loss of water is large, and in the rat it frequently equals the urinary output. This loss would appear to be mainly from the respiratory tract, and since oxygen requirements are proportional to surface rather than to weight the pulmonary ventilation of small animals must be large relative to their weight. For this reason, and because the body temperature in rats is about 2° C. higher than in man, a relatively large loss of water from the lungs is to be expected.

Thus in rats the extrarenal water loss at room temperature has averaged 1·23 g. per hour per 100 g. rat, and in rabbits it has averaged 0·29 g. per hour per 100 g. rabbit. In a single experiment on a man the extrarenal loss was roughly 0·04 g. per hour per 100 g. man.

When rats or rabbits are placed in a chamber at 37° C. the necessity for heat regulation causes an increase in the respiration rate, and as one

would anticipate an increased extrarenal loss of water. At the same time the urinary output is diminished and in rabbits there is a slight increase in body temperature. (A slight increase in body temperature was also observed in the few rats in which it was measured.) It would be a reasonable supposition that the loss of water by another channel explains the lessened output of urine; but in Part I it was shown that the rate of urine formation at any given moment was not parallel to the excess of water in the body at the time. We therefore decided to explore the possibility of some other factor being the cause of the reduced urinary output.

With this object in view a series of experiments were performed in which the animals were allowed to lose water extrarenally before giving water by stomach tube. Now although the total extrarenal loss of water was greater than normal in the rabbits and rats where the diuresis was conducted in a warm chamber (Tables VI and VIII), yet it was even greater in the above-mentioned animals which were allowed to lose water extrarenally and were removed from the warm chamber some time before receiving their dose of water. Despite the greater loss of water in these latter animals their outputs of urine were considerably larger than the outputs of the rabbits which were kept in the warm chamber while diuresis was in progress.

These results are more clearly demonstrated in the rat, in which animal the extrarenal loss is relatively great. Thus, in several experiments when, despite the addition of water per os, there is still a deficit of water in the body, a typical diuresis is obtained not appreciably less than that of normal controls.

It appeared, therefore, that it was some effect of a warm environment other than increased extrarenal loss of water that was responsible for the inhibition of renal activity. It has also been shown that this factor is not the failure of water absorption. Again, 2 hours after giving water when the urinary inhibition produced by the warm chamber appeared complete, five of the rats were transferred to a cage at ordinary laboratory temperature. A second though smaller diuresis was obtained in all rats after their removal from the warm chamber although no more water had been given. But no such diuresis was obtained in the third hour in any rat left in the warm chamber.

It is clear that if extrarenal loss alone had been the factor responsible for urinary inhibition then there would have been no second diuresis in the rats removed from the warm chamber at the end of 2 hours.

Since it has been shown that the temperature of the rabbits is increased

in the warm chamber it seems not unlikely that the increase in temperature or some factor related to it is responsible for the urinary inhibitions. In favour of this may be quoted the effect of immobilizing rabbits. We have shown in the earlier part of the paper that the temperature regulation of rabbits appears to be upset if the animal is immobilized and that on placing immobilized rabbits in the warm chamber their temperatures rise by 2 or 3° C., whereas unbound animals have increases of less than 1° C. Likewise at ordinary room temperatures the body temperature is maintained in unbound rabbits but falls a degree or so in bound rabbits.

The ordinary inhibitions produced by a warm chamber at 37° C. are much more complete in bound animals where the rise of body temperature is appreciable than in the unbound animals where the rise of body temperature is slight. The balance of evidence is rather in favour of changes in body temperature being responsible for urinary inhibition.

The fact that a normal diuresis can be obtained when there is no excess of water present in the body indicates clearly that water diuresis is not a delicate expression of the body's water needs, and that it may take place when the animal's water reserves are already depleted and cause a still further reduction in the water reserves.

Part I provides a further illustration of the absence of parallelism between the water content of the tissues and urinary output at the time, and a previous paper by one of us [Smirk, 1932] confirms the observations of Priestley that diuresis is not dependent upon the increases in the water content of blood as measured by the hæmoglobin percentage.

Water diuresis does not appear therefore to depend upon an excess of water in the blood or tissues and may actually take place when the water reserves are depleted.

Conclusions.

1. In rats and rabbits a previous reduction of the water reserves of the body fails to produce an equivalent diminution in the diuresis which follows the administration of water per os.

2. In rats a typical water diuresis has been obtained when there is no excess of water in the body.

3. In air at 37° C. rabbits and rats have a reduced urinary output and an increase in their extrarenal loss of water. The increase in the extrarenal loss of water is not mainly responsible for the inhibition of renal activity. The inhibition may be caused by the slight increases in body temperature observed under these conditions.

4. The simple binding of a rabbit alters its temperature regulation to a degree which necessitates the control of this factor in diuresis experiments.

We wish to thank Prof. T. R. Elliott for his helpful criticism and advice.

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THE RELATION OF ATHLETIC STATUS TO THE PULSE RATE, IN MEN AND WOMEN.

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Physiological literature contains a number of references to the fact that exceptionally slow pulse rates have been recorded in the case of highly trained athletes. Yet a search for precise data on the subject reveals the fact that very few observations have been made on it, and as far as the writer has been able to ascertain no data have been published giving the exact status of a series of athletes and their respective pulse rates.

The chief references to this topic appearing in the literature are given in the following brief review:

In 1909 Miss Buchanan, in writing "On the physiological significance of the pulse rate," thought it noteworthy to record the fact that a famous Oxford stroke had a resting pulse rate of 45. In the same year Michell published an account of observations on a large number of undergraduates at Cambridge who took up athletics during their University careers. He found that the average pulse rates (including early morning and late evening pulse rates) which were counted under nearly basal conditions were: first year 69, second year 64, and third year 56. When school athletes were excluded from the group the averages were: first year 74, second year 68, and third year 58.

Although these figures show conclusively that athletic training decreases the resting heart rate, the extent of the slowing may be exaggerated in these figures for a reason that is discussed at the conclusion of this review.

Michell [1909] further records that when a picked team of men, some of whom at least have been taking part in some form of exercise for more than 3 years (for example a University eight), is examined, the early morning frequency may be 52; the rate in some individuals being 48 or 46.

Dawson [1919], who observed the effect of physical training on pulse rate and blood-pressure during training, found that the resting

pulse rate was decreased after the period of training, but quotes no actual figures. In 1926 he further investigated the effect of athletic exercise on the resting pulse rate, this time in girls from 13 to 19 years of age.

In one set of observations the athletic girls showed an average pulse rate (recumbent) some 11 beats (85–74) below the non-athletic group, while in another set, the decrease in the athletic girls averaged 4 beats less per minute (84–80).

In 1927 Henderson, Haggard and Dolley, writing on the significance of rapid and slow pulse rates, state that "athletes frequently have very slow pulses, their rates are ten to twenty or even thirty beats slower than in men of sedentary habit." They give no group results, as these observations formed part of a wider investigation. In the protocols of other data however, the minimum resting pulse rate was 45 per minute.

In 1927 Schneider, Clark and Ring investigated the influence of physical training on the basal pulse rate in the case of five subjects who took up strenuous exercise for periods ranging from 4 to 16 weeks. The extent of the work was not less than 1 hour daily and included tennis, running, handball and swimming. The basal pulse rates were on an average slower after the period of training in the case of four out of the five subjects, to the extent of some 2 to 3 beats per minute.

Although the results are consistent with the previously recorded observations, the conclusions are vitiated by the fact that no account was taken of the possibility that a seasonal influence might be operating to produce a change in the basal pulse rate. That this might be an important factor to consider in observations extending over relatively short periods, is evident from the work of Griffiths, Pucher and others [1929], who found in four subjects a seasonal change in the basal pulse rate over a period of 2 years, with a summer minimum averaging some 7 beats per minute lower than the winter rate.

In 1929 Hoogewerf made an extensive series of electro-cardiographic observations on over 200 Olympic athletes. He mentions incidentally with regard to the pulse rate that the mean value was 50. No attempt was made, however, to grade or classify the athletes in any way, nor was any measure of variability indicated by standard deviation, probable error, etc. This aspect of the data was not developed, probably because no pulse rates were actually counted for the full minute, but were determined from the short period of the electro-cardiographic tracing.

It is therefore probable that Hoogewerf recognized that the record time was too short to yield accurate individual data, suitable for statis-

tical treatment, but that the mean pulse rate of all would approximate to the true value.

Contemporaneously with Hoogewerf's observations, Bramwell and Ellis [1929] conducted a clinical investigation on the same set of athletes. These workers were apparently more interested in other measurements, and although the resting pulse rates are given for the various groups of athletes, no special attempt was made to achieve really good conditions of rest.

Since, however, the athletes were all observed under approximately the same conditions it is of interest to pick out the runners from the tables and place them in a series together as follows:

Type of runner	Resting pulse rate
Sprinters	66
Middle distance runners	63
Long distance runners	61
Marathon runners	58

It will be seen that we have arranged the runners in order of the increasing duration of their athletic exercises. It is at least striking that the mean pulse rate decreases in each case in the same direction. Although the number in each group (15–30) is not large enough for inferences statistically sound, this relationship becomes more significant in view of the facts already reviewed, and of the data presented later in this paper.

Of all the data quoted in the foregoing, that recorded by Dawson [1926] on girls and Michell on men undergraduates are the only cases in which really systematic observations were made to assess the effect of physical training on the pulse rate.

Dawson's results appear quite convincing; but on account of the age and sex of the subjects (young girls), the amount of athletic training was probably but moderate, so that nothing exceptional was recorded in regard to really slow heart rates.

The same cannot be said with regard to Michell's data, for it is quite possible that part of the decrease in pulse rate in the three successive yearly tests may be due, not to real changes in the individuals, but to the experimental conditions operating in two ways, viz.:

(1) To an increasing degree of care on the part of the observer in ensuring complete rest of the subject. This is suggested by the figure 74 quoted as the mean pulse rate in the first-year series. This average is exceptionally high for conditions that include both early morning and late evening counts, even for an unselected set of men, whereas Michell's

group included a good many athletes. Judging from our own figures and those quoted in the literature one would not expect a higher mean pulse rate than about 66 under these conditions. It is therefore scarcely possible that the subjects were taken in the first year under adequate conditions of repose.

(2) To the possibility that increasing familiarity of the subject with the observer and the conditions of the experiment may make for some improvement in the resting state.

This criticism may be supported by the fact that the basal pulse rates in the case of three separate groups of medical students, taken under the same conditions at the University of Sydney, were lower in a second series of observations on the basal pulse rate made 12 months after the first. In the second set of observations the extent of the decrease in the pulse rate was 4, 1 and 2 beats per minute respectively in the three groups.

It is worth noting in passing that according to Sutliff and Holt [1925], the age gradient in pulse decrease is insignificant at this time of life, so that this factor need not be considered in Michell's results nor in the second point of criticism of them.

It would seem, therefore, that the objections advanced above could only be removed by obtaining the basal pulse rates of a group of subjects, and dividing them into sub-groups on the basis of extent of athletic training, and by reducing the effect of individual variation to a negligible amount by making the numbers in the sub-groups sufficiently large. This was attempted in a set of observations which are described in section 2 of this paper.

The same procedure appears already to have been adopted by Pembrey and his co-workers in analysing the pulse data of some 94 men and 103 women.

As the writer has not been able to obtain the original reference to discover how the division into athletic and non-athletic subjects was made, he can make no comment on the data other than draw attention to the fact that greater numbers than those observed would be necessary to stabilize the difference in pulse rate between the two constituent groups of subjects.

A summary of Pembrey's [1922] results is given by Lamb [1930] in his book on Human Physiology. The mean value for trained subjects was 61 as against 72 for the untrained subjects in the case of men, and for the corresponding groups of trained and untrained women 75 and 84 respectively.

The objects of the present communication are two in number:

(1) To present the basal pulse rate data in the case of two highly trained groups of athletes (one of which shows the lowest mean basal pulse rate yet published), and to record in this connection the exact status of each athlete on a quantitative basis.

(2) To describe an investigation of the variation in basal pulse rate in both men and women according to extent of physical training.

Section 1.

Dealing with quantitative athletic status and basal pulse rate of exceptional athletes.

It is naturally a difficult matter to obtain extensive data along these lines, for not only is the field of subjects highly restricted, but it is no easy matter to realize the conditions necessary for determining their basal pulse rates.

In the year 1921, however, an opportunity arose for obtaining these data, when a number of selected athletes, including Olympic competitors from abroad, were grouped together at the same hotel while taking part in the swimming championships of Australia. The pulse rates were counted after a night's rest, the subjects remaining recumbent in bed after waking, until the observations had been made. Several counts were made on successive mornings on each subject.

The essential data are given in Table I, together with a detailed account of the status of each athlete.

It should be noted that the group is small, since it is restricted to those having to their credit at least a championship of one of the states of Australia. In addition each athlete has at least been placed first or second in an Australian swimming championship, and two of them have each had the distinction of being the world's best middle distance swimmer of his day.

The quantitative status of each athlete is assessed by expressing his best performance (officially timed in a championship race), as a percentage of the existing world's best for the distance. For example, let T_s be the subject's best official time for a given distance and let T_w be the world's record for the same. Then, since the quality of a performance varies inversely as the time, the quantitative status is given by

$$\frac{T_w}{T_s} \times 100.$$

Ten years later, in 1931, a further opportunity presented itself for the recording of data from another group of athletes of exceptional calibre.

TABLE I. Basal pulse rates and status of a number of champion swimmers.

Initials	General status of athlete (Ol. A.) = Olympic Athlete	Quantitative status. World's record / Athlete's best × 100	Basal pulse rate: Mean	Basal pulse rate: Minimum	Weight in kg. stripped	Age in years	No. of years of strenuous athletic training
L. L.	Holder of world's record for ¼ mile (Ol. A.)	100·0	52	47	70·0	30	14
F. E. B.	Winner of innumerable championships from 220 yd. to 1 mile in Australia and Europe over a period of 15 years (Ol. A.)	99·9	50	49	65·9	30	15
P. K.	Won several Australian championships. Placed second 100 metres, Olympic Games, 1920; representative of U.S.A. (Ol. A.)	98·1	42	41	80·9	19	3
F. S. C.	Winner of ¼ mile and ½ mile championships of N.S.W. (Australia) and second in ¼ mile championship of Australia	95·6	40	38	65·3	31	12
I. S.	Won many state championships of Victoria (Australia) over a period of years. Established Australian record for 220 yd. breast stroke (Ol. A.)	94·8	53	52	72·7	30	12
M. C.	Placed second in ¼ mile championship of Australia. Subsequently won many Australian championships from ¼ to 1 mile (Ol. A.)	94·7	47	41	65·9	21	4
S. S.	Winner of several championships of Queensland (Australia) from ¼ to 1 mile	93·8	49	46	56·4	31	13
S. B.	Winner of breast-stroke and back-stroke championships of N.S.W. Winner of 220 yd. breast-stroke championship of Australia	91·3	47	43	59·1	24	5
	Mean	96·0	47·5	44·6	67·0	27	10

TABLE II. Basal pulse rates and status of highly trained middle distance runners.

Initials	General status of athlete	Quantitative status. World's record / Athlete's best × 100	Basal pulse rate: Mean	Basal pulse rate: Minimum	Weight in kg. stripped	Age in years	No. of years of strenuous athletic training
J. H. B.	Winner ½ mile championship, Sydney University, 1930. Winner ¼ mile open scratch race N.S.W. (Australia)	94·8	50	48	81·8	22	5
P. H. W.	Winner mile championship, Sydney University, 1929, 1930, 1931. Ex-holder University mile record	93·8	62	60	61·8	21	5
K. S. J.	Holder of N.S.W. junior state record for ½ mile	93·1	58	56	66·0	20	4
R. B. T.	Winner ½ mile championship, Sydney University, 1931. Holder University 1 and 2 miles record	92·2	49	48	64·5	20	4
G. R. J.	Half-mile runner. No championships to his credit	91·2	53	50	73·1	18	3
	Mean	93·0	54·4	52·4	69·4	20	4

Although only five in number these men form far the best and most highly trained group of middle distance runners ever present at the University of Sydney. The data, set out in the same way as in Table I, are given in Table II.

Comments on Tables I and II.

It is a striking fact that Bramwell and Ellis found the lowest pulse rates of all groups of Olympic athletes examined to occur in the case of the Marathon runners, where the mean age was greater than in the other group. They say "the Marathon runners are quite a distinct type. They are much older men, in the late twenties and early thirties." The mean age given for the group was 27 years. This is suggestive that the effect of exercise in decreasing the heart rate continues to operate steadily over a period of years. It will be noted that the mean age of the group of swimmers of Table I is also unusually high—27 years—five of the eight being over 30 years of age. Moreover, four of these five were then at the apex of their physical condition, inasmuch as during that year they recorded better performances than ever before.

The number of years of hard physical training is on that account much greater (10 years) than in Michell's rowing eight (3–4 years), or of the group of half-mile runners of Table II (4 years).

In explanation of the long periods of training quoted in certain cases in Table I, it should be stressed that these are quite conservative. It may be mentioned that it is not uncommon for champion swimmers to mature early[1] and to maintain their form for much longer periods than most athletes. No better example can be given than in the case of F. E. B. of Table I who at the age of 14½ years won the quarter-mile championship of Victoria, and who won in all 32 championships of Australia commencing at the age of 15 and concluding his series at the age of 33. In addition his first and last appearance as a representative of Australia and place winner at the Olympic Games, were spaced 16 years apart (1908 and 1924).

Whether or no these are the influences that have operated to produce the lowest mean basal pulse rate for a group yet recorded, it is not possible to say, as the number is too small for statistical significance. Only an accumulation of such data could settle the question.

[1] For example, a world's record has been broken by a swimmer of 15 years of age, a thing without parallel in any other sport. The same swimmer (A. Charlton of Australia) at the age of 16 won the 1500 metres race in 20 min. 6⅘ sec. at the Olympic Games (Paris, 1924), again breaking the world's record for the distance.

Section 2.

Dealing with quantitative athletic status and basal pulse rate of athletes of varied calibre.

The object of the observations now to be described was to obtain data as to the effect of athletic exercise on the pulse rate in such a way as to obviate any fallacies such as may have affected the observations recorded by Michell.

In presenting the results it is felt that still larger numbers of subjects in the groups would have been desirable, but as the data were necessarily obtained in different years and not always under the same conditions of rest or time of day for different groups, these have been kept separate. On the other hand, the fact that all groups show the same kind of change, though varying in degree, reduces to the vanishing point the possibility that the differences occur by chance.

The method of grading any one group of subjects (whose individual pulse rates were all determined under the same conditions) into various sub-groups according to their athletic history was in the first instance as follows:

A general questionnaire was prepared setting out in tabular form all the common forms of sport, recreation and athletic exercises. The subjects, who were practically all medical students (between the ages of 19 and 21 years), were required to fill in an account of their activities in each section, and to indicate the degree of proficiency attained. For example, a subject might record that he had played first grade football for two seasons, had run a mile in a certain time, and so on.

Upon examination of the forms an attempt was made to classify the subjects into four groups:

(1) Those of less athletic calibre than the average.
(2) Those of average athletic calibre.
(3) Those of rather higher athletic calibre than the average.
(4) Those of a distinctly high athletic calibre.

Although a somewhat different set of groupings might have resulted if the completed forms had been sorted by different investigators, the writer was satisfied that a reasonably good sub-division had been arrived at when all had been considered.

Certain guiding principles were employed in the assessment of the athletic histories, *e.g.* a first grade footballer, playing for several seasons, would be placed in the fourth group, whereas a second grade footballer would be placed in the third group. Again, the fact that a subject had

run the mile in something of the order of 5 min. would qualify him for group 4, while a performance of 5½ min. for the mile would qualify for the third group. Further, where several sports or games had to be considered collectively, football and rowing were regarded as more strenuous than hockey, the latter more strenuous than cricket, and so on. In the case of swimming, the specified quotation of certain times for distances was a valuable guide.

In all there were 121 subjects in this first investigation, who were grouped as described.

As the number in the fourth group was too small, it was decided to amalgamate this with group 3, and thus to compare the basal pulse rates for the three main subdivisions:

(1) Those of less athletic calibre.

(2) Those of average athletic calibre.

(3) Those of greater athletic calibre.

The results were rather unexpected, as very little difference was shown in the three groups, viz.:

Group 1.	Mean basal pulse rate	61·9
Group 2.	,,	61·7
Group 3.	,,	59·5

The writer found it difficult to understand why so small a difference as 2·5 beats per minute should appear between the extreme groups, in view of the profound decrease in the pulse rate of the group of athletes in Table I. It seemed possible that a distinct effect in lowering the pulse rate might only be produced by very intensive physical training, and that in the group examined there were too few heavily trained subjects to make much impression upon the group averages. Further, in the case of the two groups given in Tables I and II, their calibre is fixed by definitely measurable criteria which lends significance to the results in spite of paucity of numbers, whereas in the student groups errors of classification may operate to quite an unknown extent.

As the results were not encouraging the observations were abandoned, and were only revived many years later upon the consideration of a fresh point of view. In 1930 it was decided to re-investigate the matter by changing the method of grouping.

Now it often happens that a first grade footballer or a first class runner may owe his pre-eminence more to innate physical ability[1] than

[1] This disturbing factor would be negligible where athletes of the calibre of those in Tables I and II are concerned, since in these cases competition is so keen as to demand hard training on the part of the athlete.

to actual training, and may have undergone less actual physical exercise than, say, a second grade footballer or a second class runner. Wherever this occurs the grouping would run counter to the principle of sorting aimed at, and the result of such occurrences would tend to "blur" the distinctness of the groups.

On the other hand, practically every individual indulging in various games and sports comes into contact with a good many of his peers, and has every opportunity of gauging the relative extent of his own training in relation to the rest.

In consideration of this fact it was decided to attempt a fresh mode of grouping where the classification was made by the individual himself in relation to his fellows. Instead then of an elaborate questionnaire each subject was asked to consider carefully the totality of physical exercise of every description that he had ever undertaken, and to classify himself into one of the following groups:

(1) A group having had a history of less athletic exercise than the average.

(2) A group having had a history of about an average amount of athletic exercise.

(3) A group having had a history of more athletic exercise than the average.

Here again some overlapping must inevitably occur owing to absence of measurable criteria, so that any real difference would thereby be diminished. In spite of this, the first group examined in this way showed a distinct difference in the sub-groups, as did each subsequent group without exception, even when the numbers were relatively small. This was true not only of the results of the basal pulse rate determinations, but also when observations were made under daytime conditions, both

(*a*) when all the subjects of a group were rested sitting for a period of not less than 1 hour, and

(*b*) when all the subjects were taken sitting but for a short period of rest only.

The results for each group are given separately in Table III.

It should be emphasized that each individual pulse rate from which the sub-group average was determined, was itself a mean of not less than six counts on the subject, and for the most part on anything from three to six separate occasions.

It is clear, therefore, that each individual value was a reliable one, and on this account any tendency of chance variations to blur the distinctness of the group mean would be minimized.

TABLE III. Difference in the mean pulse rates of groups of subjects (males) classified according to the extent of their athletic history.

Conditions under which pulse rates were taken	No. of group	Sub-groups: Less athletic history	Sub-groups: Average athletic history	Sub-groups: Greater athletic history	Column 3 minus column 5	Mean for whole group	No. of subjects in group
Basal	1	64·7	58·9	54·6	10·1	58·4	72
	2	65·3	63·0	57·7	7·6	62·3	42
	3	66·1	63·1	56·0	10·1	61·7	37
	4	67·3	63·0	60·9	6·4	62·8	30
Mean or total	—	—	—	—	8·8*	62·2†	181
Daytime	5	74·1	72·6	65·5	8·6	70·4	50
	6	79·1	76·4	71·5	7·6	74·6	60
	7	82·5	70·0	65·1	17·4	70·5	84
	8	80·1	78·0	73·9	6·2	77·4	43
Mean or total	—	—	—	—	10·9*	71·7†	237

* It should be noted that the means of the differences in pulse rate are not simply the means of the numbers appearing in the column labelled (column 3 minus column 5), but the weighted mean of the numbers, thus allowing the larger groups to exercise their due influence on the final mean.

† In obtaining the means for all groups in the basal condition, group 1 is omitted, as it was not strictly comparable with the others, for the reason explained subsequently in the text. Group 8 is omitted from the daytime average for the same reason. Here the true resting condition was not aimed at, and the group results are therefore distinctly elevated.

Comments on Table III.

The relatively low rates of group 1 are to be explained by the elimination of the higher counts of the series of individual basal pulse rates in obtaining a mean individual figure. Every subject shows a certain tendency to vary, even on the same occasion as well as at different times under apparently the same conditions. By elimination of the higher values and retaining the lower consistent figures, the results approximate nearer to "true basal" pulse rates as distinct from "mean basal" pulse rates, which are given as the average of all determinations under the same standard basal conditions.

The remaining groups 2, 3 and 4, on the other hand, show mean basal pulse rates closely consistent with those published in the literature. Here the average of all groups is 62·2, whereas the following values are given by various writers: Korosy [1911] 63·3, Harris and Benedict [1919] 61·3, and Sutliff and Holt [1925] from personal observations and from a thorough search of the literature, 62 to the nearest whole number.

Although the means of the corresponding sub-groups are not amalgamated, owing to variation of conditions, the extreme sub-group differences (given under column 5) are averaged, since any variation in conditions of a whole group affected equally both these sub-groups.

It will be seen that the average difference under daytime conditions (10·9) is greater than the corresponding difference under basal conditions (8·8). This might, however, be expected, as the daytime pulse rate is itself distinctly higher than the basal pulse rate. When the extreme sub-group difference is expressed as a percentage of the mean for the corresponding whole group, this discrepancy largely disappears. Thus the extreme sub-group difference of 8·8 under basal conditions equals 14 p.c. of the corresponding mean for the whole series, while in the daytime series the mean figure 10·9 equals 15 p.c. of the corresponding general mean.

It was found possible to obtain but one series of observations in the case of women. In this group the resting sitting pulse rates were determined after a short period of rest (some 15–20 min.), in most instances after walking a few hundred yards. The subjects were practically all between 17 and 19 years of age. The results are given in Table IV.

TABLE IV. Effect of athletic history on the daytime sitting pulse rate in the case of women.

Less athletic history	Average athletic history	Greater athletic history	Column 2 minus column 3	Mean pulse rate	No. of subjects
90·1	84·2	82·2	7·9	84·5	80

The mean pulse rate shows the same relation to increased athletic exercise as in the case of the men, namely, a distinctly slower rate in the more athletic group.

The mean value, moreover, is approximately the same as the figure quoted in the literature for women sitting at rest, viz. 84.

For the purpose of ready comparison the various sets of data for basal pulse rates in the present section are collected together in Table V.

TABLE V. Mean basal pulse rates of men, grouped in ascending order of extent of athletic training.

Class of men	Authority quoted	Mean basal pulse rate
Less athletic history	This paper	66
Average athletic history	This paper	63
Greater athletic history	This paper and Michell	57
Superior University blues	This paper and Michell's rowing eight	53
Olympic athletes	Hoogewerf	50
Group of swimmers averaging ten years' athletic training (mostly superior Olympic athletes)	This paper	47

Making due allowance for the fact that some of the groups are few in number, it is striking how the basal pulse rate invariably decreases in the table with increasing intensity and duration of athletic training.

Summary.

1. A brief review is given, drawing attention to the prevailing agreement that slow pulse rates are characteristic of highly trained athletes, and to the need for an attempt to develop the quantitative aspect of the subject.

2. Details of basal pulse rates, together with a quantitative estimate of athletic status, are given for a group of eight highly trained swimmers (each at least a state champion). The mean basal pulse rate was 47, which is the lowest group value yet recorded.

3. An attempt to trace the relation between the slowing of the pulse rate with extent of athletic history in the case of 121 men as gauged by a questionnaire failed to yield convincing results.

4. A similar attempt showed marked differences between sub-groups of 237 men, when the basis of grading was made upon the subject's estimate of his quantitative athletic history, in comparison with his fellows.

5. Confirmatory results were obtained in the case of the resting daytime pulse rates of 80 women, who were asked to grade themselves in the same way.

In conclusion I wish to express my thanks to Prof. H. W. Davies, from whose laboratory this communication is published, for his encouragement and criticism, and to all those who acted as subjects for observation.

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[1] Quoted from Lamb.

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"POSTURAL REVERSAL" IN PERIPHERAL PREPARATIONS.

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"Postural reversal" has been defined as "the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied" [Forbes, 1922]. Magnus [1910] showed that a cat's tail can be drawn reflexly from either side towards the median plane when stimulated at the tip, the direction of the movement being determined by the initial passive posture of the tail. The reaction might, with equal aptness, be termed "movement reversal." Magnus showed that the shunting effect could be traced to the influence of the deep afferents, and was therefore tonic. Sherrington [1900] found in reflex preparations that certain stimuli would cause flexion if the limb were already passively extended and extension if it were flexed.

The object of the present paper is to show that "postural reversal" can be demonstrated in peripheral preparations with direct stimulation of motor nerves, and to suggest that as well as a central factor in the grading and co-ordination of muscular movements there is a peripheral factor, namely, the changes in receptivity caused in the muscles by varying degrees of stretch.

This investigation began some years ago in an experimental study of the muscular movements of the diaphragm [Briscoe, 1920]. Tracings were taken simultaneously from the crus and the dome, and the stretch of the two parts relatively to each other was altered by flexing or extending the spine. Extending the spine stretches the crus, flexing stretches the dome. Both in movements due to natural respiratory impulses, and in those due to electrical stimuli, it was noticed that the contraction started earlier in the crus when the spine was extended and earlier in the dome when the spine was flexed. The extent of contraction was also shown to be

related to the degree of stretch, both with natural and artificial stimuli. In a pithed animal, the phrenic was stimulated by single break-shocks of varying (but near threshold) strength. In these experiments the "threshold" stimulus was taken to be that strength of stimulus which just produced a recordable movement in the muscle. It was found that, with a just minimal stimulus, the crus did not shorten if the back was flexed, and the dome did not shorten if the back was extended. In other words, the visible response to a threshold stimulus per the phrenic nerve was found to be dependent on the posture of the body causing different degrees of stretch in the two parts of the diaphragm. This change of threshold for movement was later demonstrated in the gastrocnemius of cats and rabbits, the muscle being left in its natural relationships and stretched or slackened by fixing the foot in different positions [Briscoe, 1924]. Break-shocks near threshold value were used, and it was found that the reaction was obtained with much greater regularity when the nerve was not cut off from the central nervous system. The suggestion was made, therefore, that the threshold reaction to stretch was dependent upon the integrity of the reflex arc. Ordinary single shocks of threshold value do not produce regular minimal contractions in a peripheral preparation, but in the present paper it is shown that the visible threshold reaction to stretch can be demonstrated with great regularity, even when the nerve has been severed, if the stimuli employed resemble natural impulses more closely than the single break-shock.

Methods.

The experiments have been performed on cats, under ether and chloralose or dial anæsthesia. The double nerve muscle preparation used has been usually the tibialis-gastrocnemius pair, the muscles being left undisturbed and their contractions recorded by the movements of the foot. Sometimes the gastrocnemius-soleus combination has been pitted against tibialis and extensor longus digitorum. When necessary the movements of the individual tendons have also been recorded.

The method of stimulation employed has been that described in a previous article [Briscoe and Leyshon, 1929], namely, the regular intermittent discharges of a neon lamp. The discharges are led through the primary coil of an induction apparatus. Each nerve with its stimulating pair of electrodes is connected to one of two secondary coils, which are then placed on either side of the primary coil. The presence of a second coil makes no difference to the stimuli provided by the first coil. By use

of a key in the primary circuit, simultaneous excitation in the secondary circuits can be ensured.

Results.

If muscles are kept in their natural relationships they can be stretched or slackened by moving the bony points to which they are attached. Thus the flexor and extensor groups working the ankle joint can be stretched and slackened alternately by performing passive movements on the foot, the leg and thigh being fixed by drills. These passive changes in length are of considerable importance. The preparation is so arranged that the thigh is vertical and the leg is horizontal. No particular posture is imposed on the foot, which is free to be moved either by passive traction, or by the contractions of the muscles working over the ankle joint. The foot usually takes up a neutral position, neither flexed nor extended, at an angle of about 50 degrees or 60 degrees to the horizontal, and if alternate passive movements of flexion and extension are carried out, the foot will return to approximately the same neutral position. If, however, the foot is carried passively into a position of extreme flexion and held there for a few seconds, on release the limb will not return to the neutral position, but will remain in a position of semi-flexion. If this procedure is repeated in the opposite direction the foot, on release, will take up a position of semi-extension.

The same effect can be obtained by making brief repeated passive movements in the desired direction, the return of the limb on release not being controlled in any way. It is found that the postures of semi-flexion and semi-extension thus imposed and passively maintained may vary by as much as 30 degrees, though 20 degrees and 15 degrees is the more usual range. In old cats the range may not be more than 10 degrees. The actual angles read depend a good deal on the way the preparation is set up; what is important is the range of passive posture which can be imposed and maintained without further effort, either external or intrinsic. That these changes in passive length are not in any way dependent on the central nervous system is readily demonstrated. Before the nerves are touched the leg and foot are fixed in position by drills and passive movements are performed or postures externally imposed, first in one direction and then in another. The sciatic nerve is then cut and the procedure repeated. Severance from the spinal centres makes no appreciable difference to the range of passive posture.

The reaction is shown in the following way. The leg is denervated by

cutting the sciatic and femoral trunks. The motor branches supplying the antagonist muscles used are dissected out, and each connected with one or other secondary coil. The foot is in the neutral position, *i.e.* neither flexed nor extended. If other postures have already been imposed the neutral position can be reassumed by making quick alternate passive

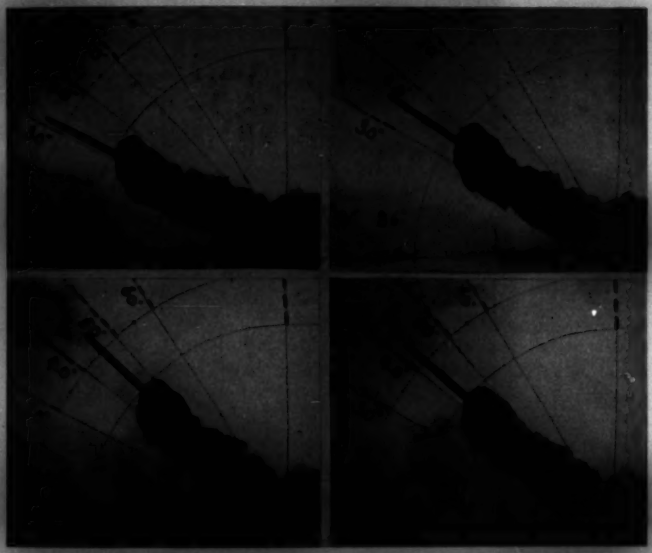

Fig. 1. The left-hand figures show the foot in the positions of passive extension (P.E.) 32°, and of passive flexion (P.F.) 46°. The right-hand figures show position of foot after the reversed movements have taken place, identical stimuli producing opposite results. Camera conditions remain constant.

movements of flexion and extension. The secondary coil used for excitation of one motor nerve, *e.g.* flexor, is placed at such a distance from the primary that a very small movement of flexion is perceptible when the circuit is closed for a brief time. This procedure is repeated on the opposing muscle using the other secondary coil. A passive posture of semi-flexion is now imposed on the foot. When both nerves are simultaneously excited, a movement of extension towards the neutral position

results. The foot is now placed in a position of passive extension. On stimulation as before, a movement of flexion takes place towards the neutral position. This is the reaction of "postural reversal," *i.e.* in each case the same stimuli are applied to the respective nerves, but the direction of movement is determined by the posture existing at the moment

Fig. 2. Record of movement of foot. Upwards extension, downwards flexion. After alternate passive movements (P.M.) foot returns to the neutral position *N*. Stimuli to flexor and extensor (signals at top) tested separately, both give small contractions in the neutral position. Primary circuit now broken and both secondary circuits closed. After holding foot in position of full extension for a few seconds, on release it remains passively in a position of partial extension (P.E.) 50°. At the arrow both nerves stimulated simultaneously by closing the primary circuit; a movement towards flexion takes place. From partial flexion (P.F.) 80°, with the same stimuli, a movement towards extension occurs. Time in seconds.

of application (Fig. 1). If the foot is now placed in the neutral position and the same stimuli applied, usually no movement takes place, though a slight movement may occur if the muscles are not perfectly balanced. Once the right conditions have been secured, this reaction can be repeated over and over again. It is more easily obtained in preparations in which there is a large range of passive posture (Fig. 2), but it

has been demonstrated where the range of passive posture was only 10 degrees[1].

If, instead of foot movement, minimal movements of the individual tendons are recorded, the same reversal of response is seen with change of passive posture (see Fig. 3).

This change in response resulting from stretch can be demonstrated in other ways than by change of passive posture. It can be shown when stretch is produced by active postural contraction of the opposing

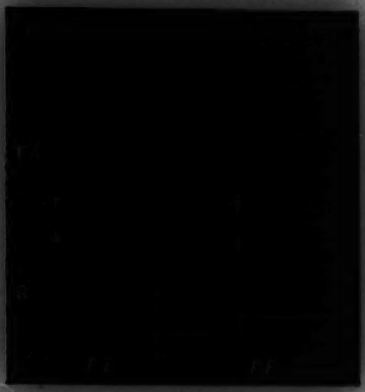

Fig. 3. Recorded from tendons. Tibialis (T.A.), median head of gastrocnemius *G*. In both cases contraction upwards. Buzzer indicates when primary circuit closed. Both secondary circuits closed. Near threshold stimuli to both nerves. When the foot is in passive extension (P.E.), tibialis only shortens and gastrocnemius is lengthened by its pull. In passive flexion (P.F.), gastrocnemius shortens and tibialis is lengthened.

muscle. This is best seen when the muscle to be stretched (gastrocnemius) is stimulated by currents which vary rhythmically in strength so that regular phasic contractions result, and the stretching muscle is stimulated by currents of constant strength causing a steady contraction. The strength of the phasic stimuli entering the extensor nerve is reduced until they are just below threshold for movement in the unstretched muscle, and there is no visible contraction. The constant stimuli entering the flexor nerve are of such a strength that the foot is drawn into a position

[1] In one pair of muscles the gastrocnemius lengthened from 121 mm. to 125 mm. in passing from a passive posture of 45 degrees to 60 degrees. The tibialis lengthened from 95 mm. to 98 mm. with the same range of passive posture.

of active flexion. This stretches the gastrocnemius, the threshold for movement is altered and the subliminal stimuli to that muscle now become supraliminal, and regular phasic extensions of the foot take place from a posture of flexion. If flexor ceases to contract and to stretch extensor, the phasic stimuli become subliminal again (Fig. 4).

Fig. 4. Movement of foot. Flexion downwards. Primary coil stationary. Flexor secondary coil stationary—submaximal stimuli of constant strength enter flexor nerve. Extensor coil approaches and recedes from primary—phasic stimuli enter extensor nerve. Movement of coil recorded in second line from bottom. Stimuli to extensor are first shown to be subliminal ↑; when the muscle is stretched by the postural contraction of flexor they become supraliminal. The short lapse in extensor excitation shows the effect of flexor alone. When the action of flexor is removed the stimuli to extensor again become ineffective, as soon as the muscle has shortened sufficiently. Time in seconds.

The change of response is best seen in flexor when that muscle is subjected to phasic stretch. This is secured by supplying the stretching muscle (extensor) with currents which vary rhythmically in strength.

The possibility that these changes in response might be due to slight movements of the nerve on the electrodes must be considered. Control experiments were done in which the nerve was deliberately slipped over the electrodes, but care was taken to see that there was no tension or pull

on the nerve terminals. This procedure, which necessarily brings fresh pieces of nerve in contact with the electrodes, does not produce the characteristic reaction. The reaction has been obtained both with the

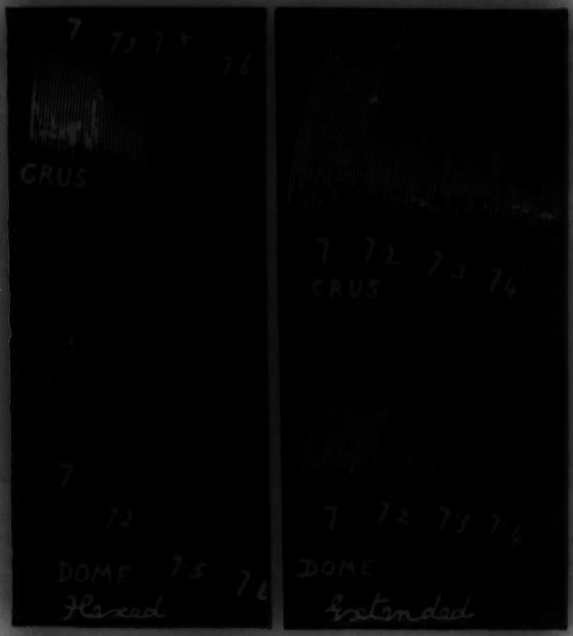

Fig. 5. Diaphragm of cat. Stimulation of cut phrenic with phasically modulated stimuli, strength reduced in small steps. Figures give distance of secondary from primary coil, coreless inductorium. In left-hand tracing spine is flexed. A near threshold stimulus (7·6) is effective in the dome and not in the crus. In right-hand tracing spine is extended. A threshold stimulus is effective in the crus and not in the dome. The sudden downward movement at 7·4 is due to a gasp of the chest muscles.

nerve lying on the electrodes without any tension and, on the other hand, with the nerve, attached by a thread to the electrode holder, under continuous tension. As a further control the phrenic diaphragm experiments have been repeated, using, instead of break induction shocks, the phasically modulated stimuli which have been shown to be capable of

reproducing accurately the deliberate movements of the diaphragm in ordinary respiration [Briscoe, 1928]. Since the nerve fibres to the crus and dome run in the same trunk, only one set of electrodes is necessary and the excitation conditions are identical, whatever the relative positions of the two parts of the muscle.

Fig. 5 illustrates this point. Records from crus and dome were obtained by means of a pneumothorax, and the muscle was paralysed by

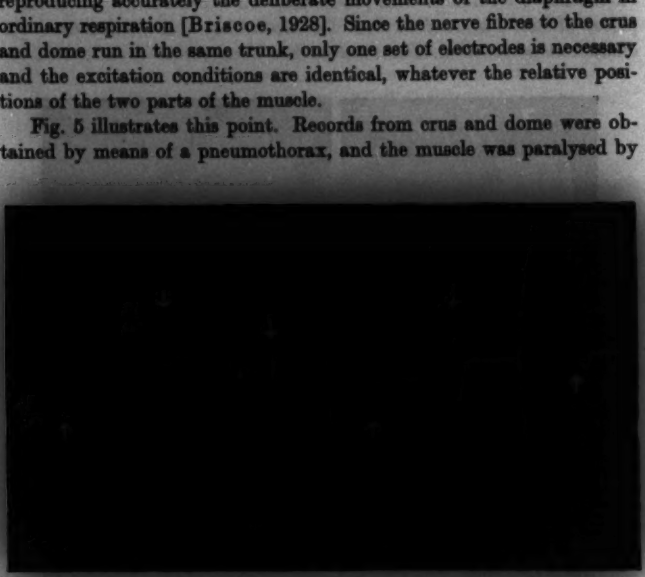

Fig. 6. Movement of foot. Ordinates, recorded on stationary drum, show arc of lever and the limits to which foot can be carried by gentle traction. Between the angles of 75° (P.F.) and 60° (P.E.) the foot remains in any position it is placed. Arrows mark the tying of ligatures on the sciatic trunk; resultant movement is dependent on initial passive posture. In neutral position, 67°, stimulus of ligature produces small double movement, in which flexion always precedes extension. Time in seconds.

section of the phrenic nerve. Phasic stimuli of sufficient strength were used to produce movements of moderate size in both parts when the animal was lying flat. When the strength of the stimuli was reduced progressively, different results were seen according to the posture of the spine. When the back was extended, the crus was stretched, and minimal stimuli were effective in producing small movements, but were ineffective in the relaxed dome. When the trunk was flexed minimal stimuli were effective for the stretched dome, but were subliminal for the relaxed crus.

Finally, the reaction of reversal of movement can be demonstrated without the intervention of any apparatus for stimulation or record. The

only dissection necessary is exposure of the sciatic in the thigh. The foot being placed in different passive postures, ligatures tied around the sciatic trunk below the hamstring branches will produce results dependent on the initial posture. Sharply defined movements of flexion or extension will be seen if the ligatures are made with a single quick tie. As many as twenty ligatures have been applied in succession to the sciatic trunk, and in each case the direction of movement in response to the stimulus was determined by the initial passive posture of the foot. The effect of six of these ligatures is shown in Fig. 6, recorded on slow and fast rates of drum. In these experiments the stimulus is applied indiscriminately to all the fibres of the sciatic, yet clean movements of reversal can be obtained.

These control experiments all suggest that the reversal of movement is due to relative changes in the receptive conditions of the muscles concerned, and not to changes in the excitation conditions at the electrodes.

Discussion.

It is well recognized that in reflex preparations there is considerable variation in the threshold value of stimuli, so much so that this characteristic has been regarded as one of the salient points of difference between conduction in reflex arcs and conduction in peripheral nerve trunks. One of the factors producing this variability of response is the condition of passive stretch in which the reflexly contracting muscle finds itself on reception of the stimulus. Liddell and Sherrington [1924] state that "initial passive stretch often renders a seemingly subliminal stimulus submaximal, thus in appearance lowering the threshold of the reflex. A single shock that produced no visible contraction in the slightly stretched muscle will on stretching the muscle a little further evoke an obvious reflex contraction."

The experiments described in the present investigation indicate that a similar apparent lowering of threshold in response to stretch takes place even in nerve-muscle preparations. Subliminal stimuli applied to a peripheral nerve trunk become effective in causing visible contraction of the muscle it supplies when the stretch of that muscle is slightly increased. When antagonistic muscles are tested by simultaneous excitation of their nerves, the different and reciprocal degrees of stretch which ensue under varying passive postures are sufficient to bring about reversal of movement in the limb.

The regular response of an extensor muscle to a constant reflex stimulus is intensified if a flexion reflex is elicited in one of the intervals

between the stimuli [Sherrington, 1906]. Such a result can be imitated in part in a nerve-muscle preparation by causing an active or passive flexion between two extensor movements. The increase in movement which can be obtained in this way is illustrated in Fig. 7. The effect, however, is not so lasting as in a reflex preparation, as there is no after-discharge, and the augmentation ceases as soon as the muscle has returned to its shortened condition.

Fig. 7. Single phasic contractions of gastrocnemius, recorded by foot movement. Lower line shows movement of secondary coil, as it approaches and recedes from the primary coil. After a passive movement of flexion, the muscle has increased initial stretch (P.F.), and starts contraction when the secondary coil reaches the point *A*. After a passive movement of extension, the initial stretch is diminished (P.E.) and contraction starts when the coil reaches the point *C*, *i.e.* when the stimuli are stronger. The two relaxation curves show practically the same time relations.

The original observation that the moment of entry of crus and dome respectively in natural respiration is regulated by the posture of the spine can be repeated in the muscles of the leg. With a given modulated stimulus a movement starts earlier when the muscle is under greater stretch than when it is under less stretch (see Fig. 7).

It may be asked if this reaction to stretch is a factor in the smooth co-ordination of opposing muscles. An analysis of co-ordinated move-

ments experimentally obtained [1929] suggests that such may be the case. Records of movements of the limb caused by each muscle acting alone are necessary, as well as records of the movements under reciprocal contraction.

It can be shown that two single movements which appear unlikely to produce a smooth double movement, yet often succeed in doing so, as the moment of entry of one muscle is regulated by the stretch produced in it by its opponent (Fig. 8). This reaction helps to explain the somewhat

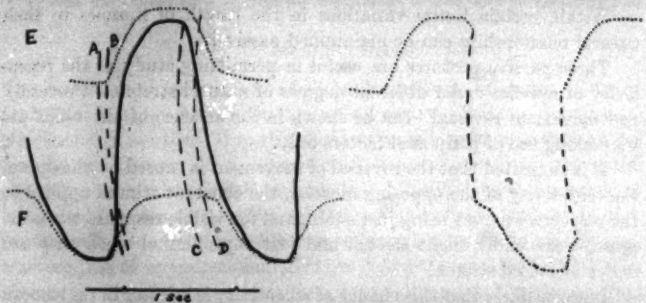

Fig. 8. Movement of foot, extension up, flexion down. Dotted lines show single separate movements of flexor *F* and extensor *E*; tracings superimposed on record of double movement (whole line) when the two muscles work together reciprocally. Time lines (broken) show arc of lever and earlier entry of muscles when stretched by their opponents. A. Start of extensor in reciprocal action. B. Start of extensor in single action. C. Start of flexor in reciprocal action. D. Start of flexor in single action. Right-hand figure shows the double movement which might have been expected from the single movements.

surprising ease with which smoothly co-ordinated movements are obtained experimentally.

The reversal of movement as seen in peripheral preparations may be due either to a balancing of smaller contractions against larger contractions, the extent of contraction in each case being governed by the amount of individual stretch, or it may be due to changes in the excitability of different muscle fibres, so that more fibres come into action with a given stimulus when stretch is increased.

It does not seem possible on the present data to decide between these two possibilities, although the observation that with a modulated stimulus a movement starts earlier in a muscle under increased stretch favours the latter alternative.

As a result of these experiments it is suggested that when a reaction of "postural" or "movement reversal" is seen in reflex preparations the mechanism is really twofold; though the central factor is responsible for the arrival of an increased number of impulses of proprioceptive origin (as the result of the stretch), yet the peripheral factor also does its part by increasing the receptivity of the muscle stretched.

Summary.

Within certain limits variations in the length of muscles in their natural relationships can be maintained passively.

These passive postures are useful in permitting study of the receptivity of muscles under different degrees of natural stretch. "Postural" or "movement reversal" can be shown in the absence of the reflex arc by making use of peripheral factors only.

It is suggested that the reversal of movement is caused by changes in the receptivity of the opposing muscles, the constant stimuli applied to the respective nerves being just subliminal for visible response when the muscles are under slight stretch and just supraliminal when they are under increased stretch.

It seems likely that this change of receptivity is a factor in the smooth co-ordination of antagonistic muscles and in the changes of reflex response to different degrees of stretch. In the intact organism this peripheral adjustment in the executant part of the reflex arc probably supplements the reinforcement provided by reflex stimuli as the result of stretch.

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RESEARCHES ON THE CONTRACTURE OF SKELETAL MUSCLE.

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I. Introduction.

The literature of contracture of skeletal muscle is extensive. Gasser [1930] has recently devoted to it a critical review. Points of importance which emerge are that (1) contractures seemingly quite diverse have yet features in common; (2) many reversible contractures in their essential chemistry resemble normal contraction. All alike show formation of lactic acid [see Gasser, 1930], splitting of phosphagen and production of ammonia. Iodoacetic acid prevents formation of lactic acid in contracture just as in contraction [Bethe, Norpoth and Huf, 1931]. The chemistry of contracture seems thus not to differ qualitatively from that of contraction.

Further, the researches of Hartree and Hill [1922] on veratrine contracture have shown quantitative similarity between the energetics of contracture and contraction. "The efficiency—measured by the ratio H/Tl—with which prolonged contraction is maintained is almost exactly the same in veratrine contraction and in normal tetanus." The same conclusion is reached by the myothermic researches of Hartree and Hill [1924, 1928], and of Furusawa and Hartree [1926] on caffein contracture and the contracture provoked by direct stimuli of greatly supramaximal intensity; also for this latter contracture by the analyses of Meyerhof and Lohmann [1925]. We seem to have before us here a broadly applicable fact.

Its theoretical importance is great. It shows that the reversible contracture of skeletal muscle is neither extreme slowing of the process of relaxation nor the operation of a special contractile apparatus, myofibrillar, or—on Bottazzi's [1897, 1926] hypothesis—sarcoplasmic.

Since reversible contractures neither in chemical process nor in mode of energy turn-over depart essentially from ordinary tetanus the differences between the two must precede the final mechanical and thermal

steps. One is led thus to approach contracture by the path of muscular excitability, especially the reversible contractures which are reactions of the muscle fibre to a stimulus, electrical or chemical.

Reversible contracture of skeletal muscle might be the result of a muscular excitation whose characteristic was slowness of development and subsidence, with absence of wave propagation. This hypothesis is borne out by the facts obtaining for direct and indirect electrical excitation of contracture, as also by the electromyogram of the "neuro-muscular" contracture of the anural Amphibia.

II. Material and general method.

The experiments have been mostly on various Anura and the cat. In the former the tendency of any given muscle, especially gastrocnemius, to respond to excitation by contracture is variable. Two factors of influence are species and nutrition. *R. temporaria* especially tends to contracture, a tendency favoured by vigorous condition of the muscle and by resistance to fatigue, therefore probably by richness in reserve nutriment. The tendency decreases under captivity.

My experiments have been on the muscles *in situ* with circulation intact (decerebrate). The contractions have been recorded, either isotonically on the kymograph with light levers (5–10 g.), or isometrically and optically with the torsion-wire type of myograph of Sherrington of rapid natural frequency, and photographed along with the electromyogram given by the string galvanometer (Cambridge new model).

III. Galvanic contracture.

If the reversible contractures of skeletal muscle connote an excitability of extreme slowness, that slowness should reveal itself (1) by high chronaxie, (2) by possibility to evoke contracture by "addition latente," *e.g.* summation of direct electrical stimuli too brief to be singly adequate, (3) also by summation of successive motor nerve impulses.

It is well known that closing or opening a galvanic circuit in series with frog muscle can excite a response which, owing to greater or less contracture, exceeds in duration the twitches excited by stimuli, indirect or direct, which are quite brief. Biedermann [1895] studied this galvanic contracture particularly in relation to the direction of the stimulating current. I have tested the influence of the duration of the electrical stimulus upon the contracture. I have excited the gastrocnemius of *R. temporaria* lengthwise by two AgCl electrodes, at tendon

and proximal pole of the muscle respectively, the contractions being registered isotonically. Under these conditions muscles tending even strongly to galvanic contracture respond by twitches free from all contracture to brief single stimuli—break shocks or feeble condenser discharges—adjusted to give contractions of comparable "height." In general the contracturing action of the galvanic current is more marked, or present only, for the "ascending" current. Fig. 1 A shows the contracture from an ascending current (cathode proximal), while a descending current of the same strength (Fig. 1 B) excites only a twitch without

Fig. 1. Galvanic contracture of the gastrocnemius of *R. temporaria* "longitudinally" excited. Isotonic lever with a load of 5 g. Make and break of current marked by arrows pointing upwards or downwards respectively. Reduced by ½. A. Ascending current (*a*) of 1·5 volts. B. Descending current (*d*) of 1·5 volts. C. Descending current of 2·5 volts. D. An experiment (performed on another preparation) showing the contracture producing action of the cathode and the inhibitory action of the anode. Explanation in the text.

contracture at closure, at opening a smaller twitch but clearly prolonged by contracture. When the current is ascending the greater part of the muscle is submitted to the contracturing action of the virtual cathode, near the proximal pole of the muscle; with the opposite direction of current (cathode at tendon) the main bulk of the muscle is under the inhibiting (relaxing) influence of the anode. In Fig. 1 C, with a strong descending current, the twitch at opening has quite the appearance of "post-inhibitory rebound." This relaxatory inhibition appears more clearly still in Fig. 1 D. First an ascending current is sent into the muscle and provokes a twitch followed by strong contracture which persists after cessation of the current. Some seconds later a descending current is sent into the muscle: its commencement causes a weak twitch

closely followed by relaxation of the contracture. This last recurs directly the inhibitory current stops. The figure shows a further example. This inhibitory effect of the anode on galvanic contracture proves the active character of this contracture. A similar anodal inhibition was shown by Biedermann for veratrine contracture.

In experiments on gastrocnemius of *R. temporaria* in the cold (11–14° C.) I have found it possible to separate to some extent contracture from contraction, and to measure the chronaxie of the former. Exciting

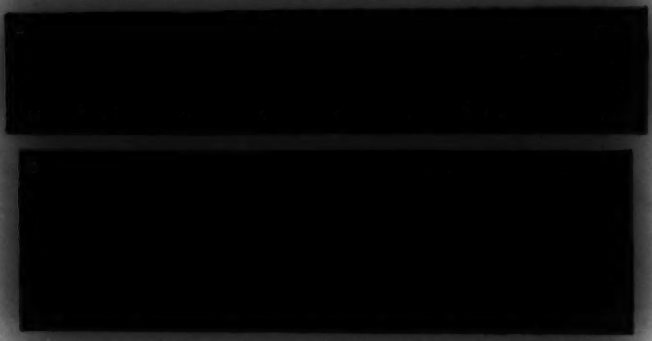

Fig. 2. Galvanic contracture of the gastrocnemius of *R. temporaria*. Isotonic lever. Load 5 g. Temp. 11·5°. Time in seconds. Reduced by ¼. A. Determination of the threshold (rheobase) of contracture. B. Determination of the chronaxie of contracture. For explanation see text. Notice the diminished height of the twitches associated with contracture. This phenomenon, which is constant, is apparently explained by a very early increase in the viscosity of the muscle, which decreases its speed of shortening and diminishes the inertia of the lever.

the muscle with currents of increasing voltage, contracture can appear regularly before any trace of the ordinary contraction (Fig. 2 A). This latter does not occur until considerably higher current intensities are reached. Hence one can measure the threshold for contracture and determine approximately its chronaxie by the well-known rapid method of Lapicque, namely, to double the rheobasic voltage and to find the shortest duration which the stimulus of doubled intensity must have in order to excite. Fig. 2 B gives a determination, at 11·5° C. The chronaxie of the contracture is 70–75 σ; that of the twitch contraction about 0·7 σ. The chronaxie of the contracture is about a hundredfold that of the ordinary contraction.

The galvanic contracture of mammalian skeletal muscle in the course of Wallerian degeneration of the motor nerve is absolutely similar to that of normal amphibian muscle (Fig. 3 B). The influence of duration of the stimulus is just as clear. It is seen in the different form of the twitch tension curves evoked respectively by the break and make shock of the inductorium. The make shock, which is slower than the break shock, evokes a twitch of sounder summit (Fig. 3 C). The twitches of the normal muscle reveal no trace of such a difference.

Fig. 3. Galvanic contracture of degenerated mammalian muscle (tibialis anticus of the cat, denervated 20 days previously). Longitudinal excitation. Sherrington isometric lever. Optical registration. Time in $\frac{1}{10}$ of a sec. Reduced by $\frac{1}{3}$. A. Galvanic excitation (ascending current of 2 volts) of the normal tibialis anticus. B. Similar excitation of degenerated tibialis anticus. C. Make-shock twitch, degenerated muscle (coil at 8 cm.). D. Break-shock twitch, degenerated muscle (coil at 8 cm.). E. Break-shock twitch, normal muscle (coil at 10 cm.). F. Break-shock twitch, degenerated muscle (coil at 10 cm.). Twitches in response to make shocks of the same height were identical in all respects.

This galvanic contracture of degenerating muscle is evidently the main cause of the seeming "slowness" of the galvanic twitch of the muscle under direct inspection, a "slowness" of diagnostic value to the clinician. Apart from this slow relaxation traceable to contracture, there exists certainly a slowness of the true twitch; this latter slowness attaches notably to the ascending phase of the isometric myograms given by degenerate muscles (cf. Fig. 3 A and E with B and F). This slowing of the twitch contraction of degenerate muscle is presumably related to the increase of chronaxie.

Discussion.

These experiments, confirming and extending older ones, show galvanic contracture as an outcome in the muscular fibre of a certain state of excitation capable of being inhibited by the anode. They define further the duration required for the stimulating electric current to give contracture. It is as though contracture attached to an excitable substance with a chronaxie much higher (× 100) than that of the excitable substance attaching to the ordinary "twitch"; in saying which I do not intend to imply the existence of two morphological entities coexistent in the muscle fibre to provide for two excitabilities. This approximate ratio 1 : 100 of the chronaxies of contraction and galvanic contracture in normal *temporaria* is just that found by Bourguignon [1923, 1931] for the two chronaxies of "myotonic" muscles of man, and by Kodera and Brücke [1928] for the two chronaxies of contraction and contracture of initial stage of veratrinization in frog's muscle.

IV. Contracture excited by direct electrical stimuli giving "addition latente."

An electrical stimulus of given intensity though failing singly, because of its brevity, to excite contraction of a slow muscle (or, generally, the reaction, of a protoplasm of high chronaxie), can yet by repetition, of above a certain frequency or length of series, be effective, in virtue of "addition latente." This summation has been the subject of researches by Keith Lucas [1907] and of Lapicque [1925, 1926]. If galvanic contracture has for its immediate antecedent a slow excitation with a very high chronaxie, one should find "addition latente," and evoke contracture by summation of brief stimuli, for example by induction shocks applied directly to curarized muscle. Previous curarization is required of course to preclude participation by nerve.

This expectation is confirmed. Gastrocnemius (*temporaria*) was employed with its marked tendency to contracture enhanced by previous short tetanization. Complete curarization an hour beforehand, 0·001 mg. per g. frog.

Electrodes at the muscle poles, cathode proximal. Break shocks from two (or three) coils, of like construction, coreless and placed so as to avoid mutual induction. The openings of the primary circuit were adjustable to 0·1σ approximately by a Lapicque rheotome modified by replacing the closure cam by an opening cam, and adding a third opening cam. The graduation was verified by means of the string galvanometer. The stimuli were slightly supramaximal for the muscle.

Under these conditions a curarized gastrocnemius, exhibiting strong tendency to contracture with two or three break shocks given directly at variable intervals, never responds to one of the shocks given singly by other than a twitch entirely like the maximal twitch to an indirect stimulus (Fig. 4 C). So also with two similar shocks separated by an

Fig. 4. Isotonic twitches of the gastrocnemius of *R. temporaria*, provoked every 2 min. Temp. 19° C. Time in seconds. Reduced by ⅓. A. Twitches showing a strong neuromuscular contracture provoked by the summation of two volleys of motor impulses at the optimum interval of 4σ. 0·05 mg. of curare was injected intravenously after the third twitch. B. Same muscle completely curarized. Contracture provoked by summation of two direct stimuli (break shocks of maximal intensity). C. Series of twitches of the same muscle showing the relation of the height of contracture with the interval between direct stimuli. D. Resistance of this same contracture to atropinization. An intravenous injection of 6 mg. of atropine sulphate between C and D.

On this figure and on the subsequent figures, single stimuli are designated by a point, and double stimuli by their interval in sigmata, under each contraction.

interval less than the absolute refractory period of the muscle measured by the least interval for tetanic summation. But, once beyond a minimal interval corresponding approximately with the least interval for tetanic summation (2σ), the isotonic twitch, now heightened because a tetanic fusion of two, is immediately followed by a secondary slow contraction (Fig. 4 B and C). The height of this secondary contraction increases at

first with increase of the interval between two stimuli up to an optimum interval of about 4σ (at 18° C.), then diminishes, first rapidly, then more and more slowly. With a greater interval than 10σ all trace of the phenomenon usually disappears (Fig. 4 C). The height of the secondary contraction can exceed that of the twitch to which it is appended. Its duration, apart from the contraction residue which prolongs it, is usually about 10 sec., but may be longer (Fig. 4 B, C, D). This secondary contraction excited by direct stimuli of appropriate interval completely resembles myographically the secondary contraction excited by indirect stimuli (*v. infra*) which is certainly a contracture.

When two successive stimuli fail to cause the contracture, for instance because too far apart, the addition of a third stimulus following the second at a similar, or even greater, interval, can evoke the contracture. If three do not suffice a greater number may. As is usual with "addition latente" an insufficient frequency of the stimuli can be met by increase in serial number.

Discussion.

It was not foreseen that two successive direct stimuli should, in order to be effective, require to be separated by an interval not less than the absolute refractory period of the muscle fibres as measured by the usual method for tetanic summation. It is as though the contracture and the contraction, though very different in their speed of excitation, were yet linked by a refractory period common to both. During the absolute refractory period the muscle, although it retains the effect of the first stimulus in the form of "excitation latente," appears insensitive to the second stimulus. The functioning of the muscle fibre is thus singularly complex.

The ineffectiveness of the single break shock for producing contracture in the above experiments seems to contradict the well-known fact that it is possible to evoke contracture of the veratrine type by single induction shocks of high intensity, or by brief rectangular currents of high voltage [Lapicque and Weill, 1912]. The contradiction is merely in appearance. The contracturing power of these brief currents is due to their intensity being such as to do damage [Suranyi, 1926]. In my experiments the short currents (induction shock, condenser discharge) were of physiological intensity and just supramaximal; they never gave contracture. I cannot then agree with Lapicque [1929, p. 105] who regards brevity of electrical stimulus as efficacious for causing contracture.

The observations of M. Lapicque and J. Weill [1912] are capable of another interpretation. In their experiments stimuli of extreme brevity, rectangular currents of 0·1σ

for the rectus anterior of *Rana*, gave contracture; the explanation lies in the considerable intensity required to be used in order to obtain twitches of appreciable height. The contracture was certainly a Tiegel contracture, as Gasser [1930] has suggested. The rectangular currents of longer duration (1–2 σ), but yet far below the chronaxie of contracture, did not give contracture because their intensity was no longer harmful, and their duration insufficient to reach (physiologically) the threshold of contracture.

V. Neuro-muscular contracture.

Of all suggestions offered to account for neuro-muscular transmission the simplest is that which likens the motor impulse in its excitation of the muscle fibre to a brief electric current. Accepting this provisionally, and admitting that the motor nerve impulse acts with a brevity comparable to that of break shock in a coreless coil, such nervous impulses, taken singly, will be as devoid of contracturing effect as are such break shocks taken singly. But when suitably repeated they will prove effective, by "addition latente."

Tiegel [1876], and after him many observers, notably Langley [1905, 1909], were struck by the impossibility of evoking a contracture by the single indirect stimulus, even of supramaximal intensity. Beritoff [1923] claims to have sometimes excited a veratrine-like contracture by a single-shock indirect stimulus to gastrocnemius (*esculenta*), the frogs having been at 22° C. for several days. I have never, myself, in hundreds of experiments, obtained a contracture in gastrocnemius from one single-shock indirect stimulus alone.

Of course, "contraction remainder," such as occurs after any shortening, active or passive, in muscles lightly weighted under fatigue, or even fresh as in the case of ileofibrilaris and coraco-brachialis of frog [Sommerkamp, 1927] is not counted as contracture. These residual shortenings, due to muscular viscosity, can, it is true, vary in the same sense as tendency to contracture; but they are not themselves true contracture, *i.e.* an active reaction. I have to insist on this distinction, since Wachholder [1931] has recently adduced as evidence of a contracture excited by a single indirect stimulus a myogram typically that of contraction remainder in coraco-brachialis. This pseudocontracture from single indirect excitation differs from the neuro-muscular contracture just described, not only myographically, but has none of its typical features, notably not the characteristic fatigability.

I find, however, perfectly possible the regular excitation of contracture in the frog by summation ("addition latente") of nerve impulses. The conditions are those given above in the previous section, with the one difference that the stimuli are applied to the sciatic nerve of the normal frog instead of to curarized muscle. The sciatic is cut at the level of the plexus and armed with Lapicque electrodes (Ag—$AgCl_2$) half up the thigh, cathode distal.

The slow "secondary contraction" provoked by two successive in-

direct stimuli at appropriate intervals is just like that provoked by two direct stimuli. The height of the isotonic myogram is similarly a function of the interval between the stimuli (Figs. 5, 6, 7 and 11). The slow contraction as a rule appears (Fig. 5 C) so soon as the interval exceeds the

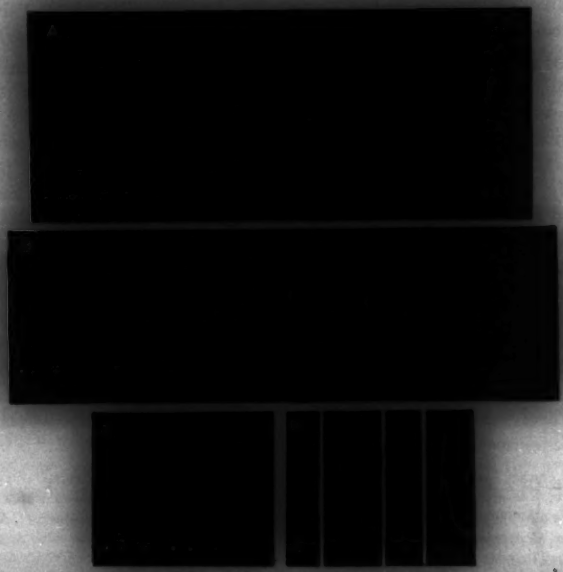

Fig. 5. Neuro-muscular contraction (isotonic) of the gastrocnemius of *R. temporaria*. Time in seconds. Reduced by $\frac{1}{4}$. A. Nerve and muscle at 18° C. B. Nerve at 18°, muscle cooled to about 10° C. C. Another preparation showing the coincidence at about one ten-thousandth of a second, of the minimum interval for summation of contracture with the minimum interval for tetanic summation. D. Another preparation showing the contracturant efficacity of three successive stimuli, when two are ineffective because of too great an interval (10σ).

absolute refractory period of the motor nerve fibres ($\pm$ 1·5σ at 18° C.). Its height increases rapidly with increase of the interval up to 3–4σ, then gradually declines. Usually all trace is lost at intervals above 10σ (Figs. 5 A and D, 7 and 11). The rise to the optimum (Fig. 6) recalls that of the curves of impulse summation (*addition latente périphérique et*

centrale), which I have described and analysed elsewhere [Bremer, 1927, 1929*a*; Bremer and Homès, 1932].

The position of the optimal interval depends on the temperature of the muscle (and neuro-muscular junctions) and is independent of the temperature of the nerve at the seat of stimulation (Figs. 5 A and B, and 6). Conversely, the minimal interval for summation depends, *ceteris*

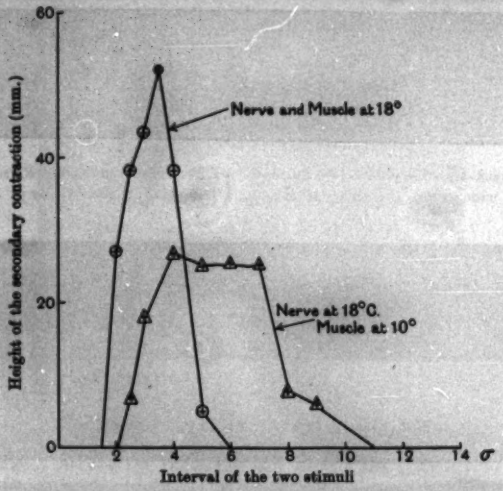

Fig. 6. Summation curves for neuro-muscular contracture constructed from the series of twitches A and B of Fig. 5. Notice the displacement toward the right of the optimum interval for summation, resulting from cooling the muscle (curve with triangles).

paribus, on the temperature of the nerve. As usual with "addition latente" it is possible to compensate excessive separation of the stimuli by adding to the serial repetitions (Fig. 5 D).

Myographic characters of neuro-muscular contracture.

The features of the slow "secondary contraction" prove it a contracture and not a contraction of special muscle fibres. Thus, it can be added to the residual contractions of fatigue or to acetylcholine contracture. In some instances I have succeeded in exciting strong contracture in a perfectly fresh muscle. There (Fig. 7) the line of the myogram joined the

abscissa in some seconds without trace of permanent residual contraction. Conversely, when excited in a muscle which has previously for a short time been tetanized, it revives somewhat the residual contracture (of fatigue), and there occurs a progressive rise of the abscissa when a succession of twitches followed by secondary contraction are evoked (Fig. 11 A). It is the secondary contraction which revives the residual

Fig. 7. Neuro-muscular contracture (isotonic) of a fresh gastrocnemius of *R. temporaria*, without any preliminary tetanization. Temp. 17° C. Reduced by $\frac{1}{4}$.

Fig. 8. Acetylcholine contracture and neuro-muscular contracture. (Gastrocnemius of *R. temporaria in situ*, acetylcholine injected intravenously.) Neuro-muscular contracture accentuates acetylcholine contracture when the latter is in the ascending phase, while it accelerates the annulment of alcaloidal contracture in the regressive phase even more than do ordinary twitches. Reduced by $\frac{1}{4}$.

contracture because simple twitches and tetani not followed by secondary contraction do not do so (Figs. 5 and 11). In the same way as intercurrent neuro-muscular contracture hastens the onset or increases the intensity of acetylcholine contracture (Fig. 8).

The height and shape of the isotonic myogram of the secondary contraction vary from preparation to preparation, but for the same experiment are constant. The height of the secondary contraction in a muscle lightly weighted (5 g.) can much exceed the height of a brief tetanus (Figs. 5 A, 9 A and 10 C). Again, the secondary contraction can graft itself

so early upon the twitch that it appears merely as an increase of height and duration of this latter. In general its form is that of a slow smooth twitch lasting several seconds (Figs. 4 A, 5, 6 and 7). At times the relaxation is late and irregular (Fig. 9 A); in such cases inspection finds the muscle bunched up towards its proximal (neural) pole and fibrillating more or less markedly.

The tension of the secondary contraction is always less than that of the twitch which precedes it, even when the shortening of the contracture

Fig. 9. Twitches followed by a secondary contraction and single maximal contractions isotonically recorded (A), and isometrically recorded (B), at an interval of a few minutes. An extreme example of the contrast existing in neuro-muscular contracture between the degree of shortening of the muscle and the feebleness of the tension developed by the contracture. Time in seconds. Reduced by $\frac{1}{4}$.

(isotonically registered) exceeds that of the twitch (Fig. 9). The strongest secondary contraction I have registered has not exceeded $\frac{1}{3}$ tension of the corresponding double twitch. This disparity between shortening and tension is often met with in contracture both of skeletal and of smooth muscle. It is important to bear this in mind in relation to the metabolism of contractures such as acetylcholine contracture. A contracture which is strong to judge by the isotonic record of a lightly weighted muscle may develop little contraction tension, and give correspondingly little lactic acid or heat. This has to be remembered of the recent experiments of Miura [1931] and of the interpretation he gives.

Fatigue and temperature on neuro-muscular contracture.

Neuro-muscular contracture is incomparably more sensitive to fatigue than is the neuro-muscular twitch. A rapid succession of twitches, each with its appended secondary contraction, soon tires out the latter

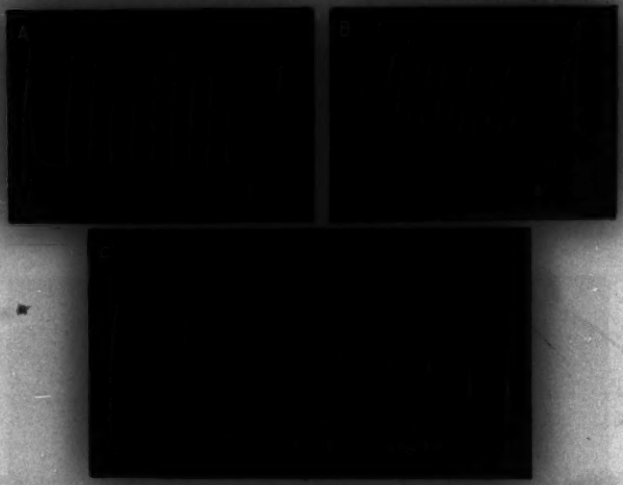

Fig. 10. A. Fatigability and very rapid restoration of neuro-muscular contracture. Having disappeared completely after a rapid series of 10 contractions (double tetani at an optimum interval of 4σ), it reappeared with the same intensity after a rest of 2 min. B. Lesser fatigability of contracture provoked by the summation of two direct stimuli on a curarized muscle. C. Selective sensitivity of neuro-muscular contracture to atropine. Double tetanic contractions (optimum interval 3·5σ) elicited every 2 min. Two successive intravenous injections of 4 and 2 mg. Atropine sulphate considerably weakened the secondary contraction without diminishing the height of the twitch. On the three records, time in seconds. Reduced by $\frac{1}{4}$.

(Fig. 10 A). Some minutes of rest restores the contracture fully. The fatigue seems mainly seated at the neuro-muscular junction, for contracture by summation of direct stimuli (Fig. 10 B) is more resistant. Besides fatigue of neuro-muscular contracture ensues quicker from twitches followed by contracture than from twitches not so followed. The mechanism of neuro-muscular contracture is therefore fatigable through the action of rapid contraction but still more so through its own action.

Warming of the muscle to 25° C. or higher annuls at once its tendency to neuro-muscular contracture (Fig. 11 A, B), but the contracture response to direct galvanic stimulation persists. Cooling the muscle restores ability for neuro-muscular contraction (Fig. 11 C).

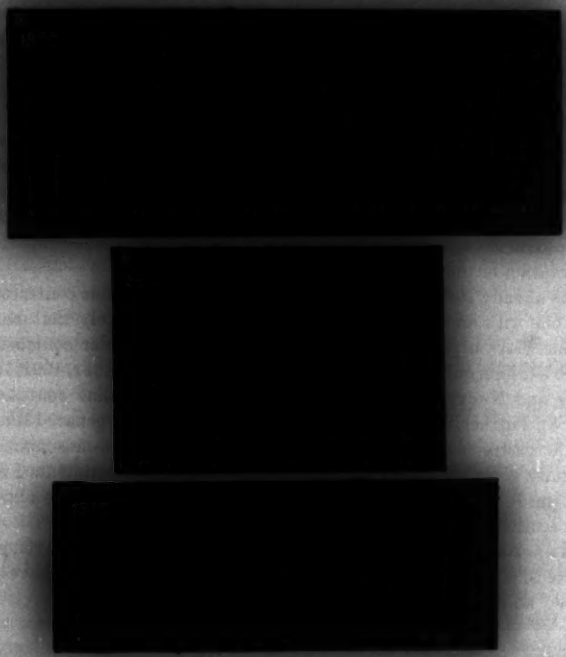

Fig. 11. Reversible disappearance of neuro-muscular contracture by warming the muscle to 28° C. The three series of twitches were successively recorded in the course of half an hour. Time in seconds. Reduced by ¼.

Pharmacology of neuro-muscular contracture.

Contracture is somewhat selectively affected by curare. Under incomplete curarization the elicitation *via* the nerve of the rapid twitch is always less impaired than is that of the "secondary contracture" (Fig. 4 A). This is intelligible, since the production of the contracture,

involving a summation of impulses, is more complex and thence more vulnerable than that of the neuro-muscular twitch. At the stage in which contracture is abolished the twitch is however much reduced (Fig. 4 A).

The atropine group has a markedly selective action on neuro-muscular contracture, even in doses which do not touch the twitch (Fig. 10 C). The atropinized muscle still responds by contracture to suitable direct stimuli (Fig. 4 D). There seems in fact a true "curarization" of the contracture, a point of interest for its explanation. The dose of atropine abolishing neuro-muscular contracture is much higher than that suppressing the parasympathetic effects. This action of atropine is probably unrelated to its parasympathetic affinity. Suffice it here to cite the identity of refractory period of the "motor" fibres of contracture and of ordinary motor fibres (Fig. 5 C). Further, the lack of proof, anatomical and pharmacological [Dale and Gasser, 1926], of any parasympathetic innervation of the skeletal muscular fibre.

As already stated the neuro-muscular and acetylcholine contractures sum one with another (Fig. 8). It is, however, little likely that acetylcholine plays a part in the production of neuro-muscular contracture, which exhibits only exceptionally the "resolution" [Riesser, 1921, 1925; Hess and Neergaard, 1924] so characteristic of choline contracture (Fig. 8). Further, acetylcholine in the perfusion fluid (oxygenated Ringer) for the gastrocnemii when they are giving strong neuro-muscular contractures gives me wholly negative results in the five experiments I have performed. Hearts of *R. temporaria* used as reagents for the alkaloid were sensitive to dilutions of acetylcholine of 10^{-7} to 10^{-9}.

Adrenaline, even in toxic doses (0·01 mg. per g. intravenously injected), is without effect on neuro-muscular contracture. I have performed no experiments using ergotamine.

Discussion.

To obtain a contracture of skeletal muscle by indirect excitation, it is thus necessary to make use of the mechanism of summation of nervous impulses by repetitive stimuli. This neuro-muscular contracture furnishes a myogram exactly similar to that of the summation of two or more brief stimuli applied to the same muscle after curarization. These facts are in perfect accord with the hypothesis that reversible contracture of skeletal muscle is the reactional expression of a very slow, specific excitability of the muscle fibre.

The remarkable pharmacological properties of neuro-muscular contracture seem to me to furnish a new argument in favour of this conception.

As we have seen, there is a manifest difference between the action of curare and of atropine on slow secondary contraction, in respect of their selectivity: when applied to the gastrocnemius of *R. temporaria*, atropine and scopolamine paralyse selectively neuro-muscular contracture, while the action of curare is on the contrary but very imperfectly selective.

In the theory of Lapicque [1926], taken in its most general form, curarization of a neuro-muscular apparatus is considered to result from the rupture of the minimum accord necessary for neuro-muscular transmission between the respective speeds of excitability (chronaxies) of the muscle and the nerve. This theory of curarization could be further enlarged by attaching it to the general hypothesis of Herman-Cremer-Lillie, and expressing it thus: The abolition of nervous command over a muscle results from all alterations of the excitability of the muscle fibre which render it inexcitable by those particular stimuli constituted by the action currents of the nerve. This alteration of excitability may be either a modification of the chronaxie, or a simple rise in the threshold of the muscle fibre.

It is well known [Lapicque, 1926] that atropine and the alkaloids of its group are much more toxic for slow muscles than they are for rapid muscles, while exactly the opposite is found in the case of curare. This explains why atropine, which has but an imperfect effect on rapid muscles, has in sufficient doses an effective curarizing effect on slow muscles. Its selective curarizing action on neuro-muscular contracture is thus well explained by the hypothesis that this contracture is the manifestation of a very slow excitability which is as such very susceptible to atropine. The possibility of this selective curarization of contracture may be considered a proof of its long chronaxie. The reality of a specific toxic action of atropine on the excitable substance of contracture is further demonstrated by the fact that this alkaloid, when in a much higher concentration than that necessary for paralysing neuro-muscular contracture, also causes the disappearance of galvanic contracture in the muscles of Anura, without nevertheless abolishing the ordinary rapid contractility [Rückert, 1930 *b*].

VI. Electromyogram of neuro-muscular contracture.

The electromyographic study of neuro-muscular contracture is of particular interest. The experiments that have just been described tend to show that reversible contracture of skeletal muscle is the expression of a specific type of excitation of the muscle fibre. At present it seems well established that the electrical manifestations of muscular contraction are, in part at least, determined by the state of excitation of the muscle fibre, independently of the mechanical phenomenon of contraction. Bishop and Gilson [1927], confirming the older observations of Lee [1887], have shown, in fact, that the monophasic electrogram of the isometric twitch of the frog's sartorius is the expression of an electrical negativity lasting during the entire period of contraction, but that this electrogram might be dissociated to a certain extent into two successive phases: (1) a brief initial negative phase of great amplitude; (2) a second negative phase, normally more or less fused with the first, the shape of which corresponds to that of the isometric myogram. According to Bishop and Gilson, the first phase is the electrical expression of the wave of excitation passing over the fibres, while the second corresponds to the electrochemical reaction which is supposed to be the basis of the process of contraction. As Gasser [1930] suggested, one can equally well account for this division of the electromyogram into two successive elements by assuming that the muscle fibre in a simple contraction passes successively through two distinct, although causally linked, stages of excitation: (1) the wave of excitation and of negativity which is translated into the initial sharp deflection, and (2) the state of excitation which is the immediate cause of the contraction, and which is shown by the delayed phase of negativity.

If this dualistic interpretation of the electromyogram is correct, then reversible contractures, which are characterized by the absence of propagation of the contractile process along the fibre, should present as their sole electrical manifestation only that negativity which is in immediate causal relationship with the contractile process and the intensity and duration of which vary proportionally as the intensity and duration of the latter. This functional negativity ought to evince itself, in the monophasic electromyogram, in a curve devoid of oscillations, whose shape corresponds more or less to that of the isometric myogram of the contraction, but which may be initiated before the appearance of tension.

This is essentially what has been observed in the case of many reversible contractures. But these potentials have been very differently

interpreted: in some cases as the electrical manifestation of an authentic state of excitation, in others as a current of deformation, and in still others as a current of alteration resulting from the harmful action of the chemical agent producing contracture [see literature in the review of Gasser, 1930].

Further, the presence of rapid oscillations complicating a continuous deviation in the electrogram of various contractures, notably of veratrine contracture, has often been interpreted, recently by Wachholder [1930 *a*], as the proof of the fundamentally tetanic nature of these contractures. To these conclusions Dittler and Freudenberg [1923] and Gasser [1930] have raised serious objections.

All these electrographic studies were made, besides, on chemical contractures, capable of causing changes in the muscle which in themselves might generate differences of potential. Thus it was particularly interesting to make an electromyographic study of a contracture provoked by electrical stimuli, especially indirect stimuli, which joins to the advantage of being the most physiological method, that of eliminating all possibility of an electrical artefact due to the stimulus.

Beritoff and Woronzow [1926] have already studied the electrical manifestations of the post-tetanic contracture of the gastrocnemius of the frog indirectly faradized. They demonstrated the existence of a non-oscillatory negativity concomitant with the contracture, which is gradually dissipated in the same time as the latter. But it is probable, because of the conditions (prolonged tetanus) and the object (*R. esculenta*) of their experiments, that the contracture was one of fatigue and not the contracture by excitation in which we are interested.

The neuro-muscular contracture of *temporaria* lends itself well to electromyographic registration because of its constancy of reaction, which permits the successive registration, in a few minutes, of twitches followed by a secondary contraction, and of ordinary single twitches.

These researches were performed by means of a Cambridge string galvanometer. Because of the small size of the electromotive forces involved the tension of the string was in general reduced to a displacement of 30–40 mm. per millivolt, which, considering the optical amplification and the resistance of the string according to the formula of Forbes and Ray [1923], represented a sensitivity of 330–450 metres per ampere. The leading electrodes were two fine silver chloride pins, one of which was inserted into the belly of the gastrocnemius muscle, and the other into the tendon of Achilles, which was itself attached to a Sherrington isometric myograph arranged for optical registration. The

resting current, generally feeble (several millivolts), was always compensated.

My own observations of the electromyogram (Pl. I) of twitches free of contracture registered under these strictly isometric conditions, with approximately monophasic derivation, and with a high string sensitivity, find that it is composed of two successive and clearly distinct phases, fully confirming the results of Bishop and Gilson [1927] mentioned above. In the case of double tetanic contractions, provoked by two stimuli at a sufficiently long interval, the positive appendage of the initial negative deflection is double (Pl. I C), while the delayed negative deflection remains simple corresponding to the myogram of the two fused contractions.

This duality of the monophasic electromyogram of a twitch, recorded with a galvanometer having a very sensitive string, corresponding to that which Bishop and Gilson [1927] obtained with the cathode-ray oscillograph in their study of the monophasic myograph of the isometric twitch of the frog's sartorius is, seemingly, as these authors suggested, the expression of a duality of origin of the potentials. The brief initial phase represents, according to this hypothesis, the potential of the muscular "impulse" which precedes the contraction; the second phase, of lower potential, and which is synchronous with the development of tension, would be intimately associated with the process of contraction. Control experiments permitted the exclusion both of the phenomenon of De Meyer (current of deformation), and of an artefact resulting from displacement of the electrodes. Pl. I D shows that passive variations in the tension of the muscle, although greater than the active variations corresponding to contractions A, B, and C, cause but insignificant variation in potential when compared with those of the delayed phase of the electromyogram of these twitches.

The fundamental experiment destined to demonstrate the electrical sign of neuro-muscular contraction consisted in recording, in a rapid series, the isometric myogram and electrogram of four twitches, some (the 1st and 4th, Pls. II and III A and D) followed by the slow secondary contraction because they were promoted by two indirect stimuli of adequate interval ($\pm 3{\cdot}5\sigma$), and others (the 2nd and 3rd, Pls. II and III B and C) free of contractures because they were provoked by a single stimulus, or by two stimuli at a sufficiently great interval (in general 10σ). As in my preceding experiments, the tendency of the muscle toward neuro-muscular contracture was augmented by a short tetanization preliminary to the rest of the experiment.

Plate I

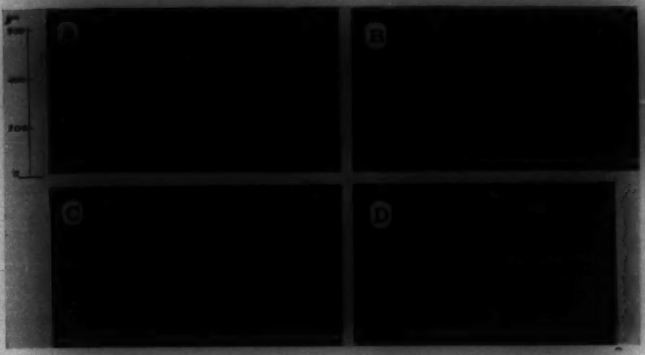

Plate II

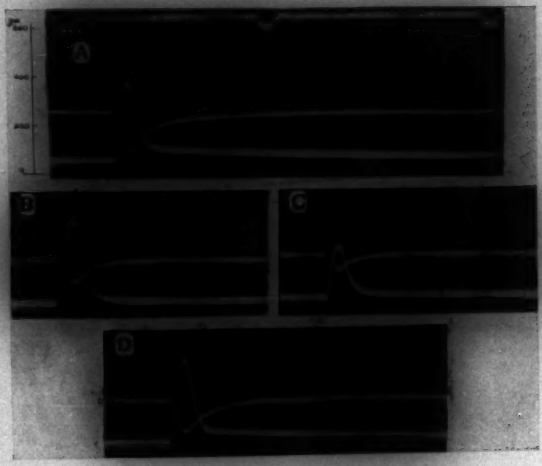

Plate III

The neuro-muscular contracture is represented on the isometric myogram (Pls. II and III A and D) by a renewal of the contraction which interrupts at various points the phase of relaxation of the double tetanic contraction, and which lasts, although progressively decreasing, for 1–2 sec.

Electrographically (Pls. II and III A and D) the contracture is characterized (1) before any mechanical manifestations by the augmentation of the amplitude of the delayed negative wave of the electrogram of the twitch; (2) by the prolongation of the decreasing phase of this wave in a curve which slowly approaches the line of isoelectricity at the same time as the line of the myogram approaches the position of initial tension of the quiescent muscle. The parallel between the two curves is striking. Sometimes the line of the electrogram of the contracture is irregularly scalloped (Pl. III A and D). These waves may pass the line of isoelectricity, especially near the end of the contracture, and give the appearance of a diphasic electrogram. In general, with the exception of these apparently secondary waves corresponding to the waves in the myogram, the curve is simple. But from time to time one can distinguish very fine rapid oscillations which are hardly visible. The appearance of the electromyogram of contracture is somewhat variable from one experiment to another, but is remarkably constant for the same muscle and for the same position of the leads.

The negative potential associated with neuro-muscular contracture is slight, as is the tension developed by the contracture, and is at all times proportional to this tension. The highest electromotive force that I have recorded is of about 3 millivolts; it corresponded, in this experiment, with a tension of 40 g., while the tension of the contraction which it followed was 300 g.

Discussion.

From these observations it seems that the monophasic (from muscle-tendon leads) electromyogram of neuro-muscular contracture of the gastrocnemius of *R. temporaria* consists essentially of a non-oscillatory negativity of the active lead. This negativity, being neither current of deformation, as demonstrated by control experiments, nor a result of alteration of the muscle substance, because the contracture was provoked by indirect stimuli, can be legitimately considered as the electrical expression of the specialized type of muscular activity represented by contracture. This potential is feeble, as is the tension developed by the contracture itself, is proportional to this tension and is slowly dissipated in the same as this latter.

The small rapid oscillations sometimes present in the electromyograms are seemingly the electrical sign of fibrillations which are visible to the naked eye in the muscle in contracture.

These observations and these interpretations are in complete accord with electrographic observations which have been recently made on other reversible contractures in skeletal muscle, especially with those of Schäffer and Licht [1926 *a*, *b*] on acetylcholine contracture and the Vulpian-Heidenhain phenomenon, and of Bishop and Kendall [1929] on aldehyde contracture of the frog's sartorius. Shäffer and Licht demonstrated the complete parallel between the electrogenic activity of acetylcholine and its contracture producing action (experiments on the mammalian muscle, in a normal state or in a state of Wallerian degeneration), while Bishop and Kendall discussed, and rejected categorically, the opinion of Verzár and Peter [1925], which attributes the potential changes in aldehyde contracture (which is of the veratrine type), to alterations contingent on the form and electrical resistance of the muscle in contracture.

My experiments, performed under strictly isometric conditions which in themselves exclude the above interpretation, reveal an additional fact, irreconcilable with that interpretation, namely, that the negativity associated with the contracture commences several hundredths of a second before the appearance of the tension on the simultaneously recorded isometric myogram. One sees in Pls. II and III that the amplitude of the negative phase corresponding to the period of relaxation of the twitch is clearly greater in the record of twitches A and D, which are followed by a contracture, than in twitches (single or double) in which contracture is absent. This increase of negativity does not correspond to any increase in contractile tension, for the tension developed by neuro-muscular contracture never determines an increase in the height of the isometric twitch. Yet it is probable (see Fig. 2 B) that the period which immediately precedes the appearance of the contracture is already characterized by an increase in the muscular viscosity.

General discussion and conclusions.

The aim of these researches was to verify the hypothesis, already deduced by a critical examination of known facts, that reversible contracture of skeletal muscle is the expression of a particular type of excitation of the muscle, which is characterized by the slowness of its development, and especially of its dissipation.

In favour of this conception I may claim to have brought forward new arguments, some indirect, and others more direct. I have been able to show: (1) that conformable to the well-known law relating the duration of the process of excitation in a tissue to the speed of excitation of the same object, to elicit a reversible contracture requires either a single stimulus of a considerable duration, or the "addition latente" of two or more stimuli which are singly too short to be effective; (2) that by using particularly sensitive muscles, such as the gastrocnemius of *R. temporaria*, one can obtain a true contracture by indirect stimulation if one takes advantage of the phenomenon of the "addition latente" of nervous impulses. I have indicated that the similarity between this "addition latente" of nervous impulses, and the "addition latente" of direct electrical stimuli of very short duration, results evidently from the fact that the bioelectric currents of the nerve impulse have the same effect on the muscle as very brief electrical stimuli; (3) a third and direct argument was furnished by a study of the electromyographic properties of neuro-muscular contracture. This contracture, provoked by truly physiological stimuli, independently of any pharmacological action, is shown to be characterized by a non-oscillatory negativity, the curve of which follows closely that of the contractile tension developed.

This particular form of excitation of the muscle may be supposed to set into action the same contractile mechanism as is employed in ordinary contraction. The identity of the metabolism of contracture and contraction demonstrated by Hartree and Hill obliges us to acknowledge the unity of the contractile mechanism. There is thus a duality of excitation but not a true duality of contraction. Contracture and contraction then, seem only to differ in the nature and duration of intermediate processes constituting the protoplasmic excitation, which form a link in the chain of phenomena leading from the stimulus to the liberation of muscular energy.

This conception of the mechanism of contracture approaches in detail that which was more or less explicitly developed by Jensen [1914], and by A. V. Hill [1926, p. 76]. That of Jensen in particular postulated, as Gasser [1930] also emphasized, "two modes of approach to the same metabolism or mechanism."

All the evidence indicates that the "excitable substance" of contracture co-exists in each individual muscle fibre with the excitable substance of rapid contraction, and is not provided for by special fibres. I have shown, in fact, that the nerve fibres responsible for the transmission of the "motor" impulses of neuro-muscular contracture are in

all probability the ordinary motor fibres. On the other hand, if it is true that different muscles of the same vertebrate can be differentiated on the basis of their unequal aptitude for contracture, and that one can even distinguish in certain muscles, as in the ileofibularis of the frog studied by Sommerkamp [1927], two groups of fibres, both on the basis of their contractile properties and their macroscopic appearance, it is also true that these fibres with particular aptitude for contracture have by no means lost their aptitude for ordinary tetanic contraction.

In a muscle capable of contracture the very same fibre can thus react according to the qualities[1] of the direct or indirect stimulus, either by a twitch or tetanus free of all contracture, or by a contraction followed by a contracture.

What is the biological significance of this dual excitability? Has it an important functional significance? *A priori* this seems hardly probable by reason of: (*a*) the extreme variability of the tendency toward contracture in the same muscle, as the gastrocnemius of Anura, according to the species, and even the individuals of the same species; (*b*) the fact that contracture of skeletal muscle does not represent a more economical mode of activity than normal contraction; (*c*) the feeble contractile tension developed by neuro-muscular contracture. No one any longer defends the hypothesis of a direct relationship of contracture with postural tonus of vertebrates.

Nevertheless, the idea of a functional rôle for this type of muscular reaction has reappeared in several recent publications. Thus Hess and Büsch [1927] have suggested its adjuvant rôle in tetanic fusion.

To this hypothesis there is the objection resulting from my own researches that at a frequency necessary for neuro-muscular contracture tetanic fusion is already complete. Besides, A. V. Hill and his collaborators have demonstrated that completion of tetanic fusion is economically provided for by the effective mechanism, common to all Vertebrata, of the prolongation of the phase of relaxation of a contraction by fatigue and by initial extension of the muscle.

Wachholder [1930 *b*, *c*, 1931], demonstrating the parallel existing between the sensitivity of skeletal muscles to contracture by acetylcholine and their degree of postural activity, attributes to this fact a fundamental biological importance.

[1] I call attention here to the fact that that which determines the appropriateness of a stimulus for a "slow" excitable substance is its relative duration, and not, as Bethe, Fraenkel and Wilmers [1922] mistakenly supposed, the progressiveness of its establishment. The rapidity of establishment of a stimulus always favours its efficacity, but slow tissues tolerate a more gradual establishment of a stimulus than do rapid tissues [Lapicque, 1926].

Since mechanical, thermal, and chemical stimuli are always of considerable duration in comparison with the duration of an induction shock, their efficacity in provoking contracture can readily be explained.

But the recent researches of Freund and Rückert [1930] and Rückert [1930 *a*, *b*] lead to a different interpretation of these "tonic" properties of skeletal muscle. They have shown that the aptitude of a muscle to electrical or alcaloidal contracture varies inversely as the ontogenetic and phylogenetic evolution of the muscle, other conditions being equal. One can discover in the adult the stages of this evolution, either in comparing in the same animal muscles of different phylogenetic antiquity, or in following the same muscle through the vertebrate series. This evolution may be accelerated because of functional causes, as in the case of the wing musculature of birds, and of some of the muscles of the posterior limbs of Anura [Freund and Rückert, 1930]. The same cause, participation in rapidly alternating movement of which the execution would be hampered by contracture, seems to me to explain the striking difference in aptitude for contracture between the gastrocnemius of *R. temporaria* and *Bufo* which are terrestrial Anura whose locomotion is chiefly by walking, and the same muscle of *R. esculenta* which is a swimming and jumping animal (Fig. 12). In this sense was taken the statement of Freund and Rückert, contrary to the interpretation of Wachholder, that "only the loss of the tonic properties seems to be dependent on the functional specialization of the muscle."

Aptitude for contracture would thus characterize a primitive evolutionary state of the muscle fibre, a condition which has been passed by some muscles but not yet by others, either because despite their phylogenetic antiquity they have retained primitive function (ocular muscles of mammals, Duke-Elder [1930]), or because, by reason of a too recent appearance in phylogeny, they have not yet had time to accomplish their evolution (diaphragm of mammals).

This evolutionary stage is also reproduced by all mammalian muscles in the course of their ontogeny [Rückert, 1930 *a*]. Normal muscles of adult mammals have practically lost these "tonic" properties, but not entirely, as is demonstrated by the greater duration of galvanic contractions compared with contractions of the same height provoked by short stimuli or indirect stimuli (compare A and F in Fig. 3), and by the possibility of provoking galvanotonic contracture by very intense stimuli. The tonic properties reappear in various pathological conditions: Thomsen's disease, myotonic myopathies, and Wallerian degeneration, which are characterized by a regression to an embryonic structural type. I have elsewhere called attention to the analogies between myotonic contracture in human pathology and neuro-muscular contracture in the Anura. This comparative study has led me to the conclusion that the myotonic rigidity which prolongs contraction in Thomsen's disease and myotonic myopathies (Fig. 13) is a neuro-muscular contracture of abnormal muscles, provoked by a summation of normal nerve impulses [Bremer, 1929 *b*; Bremer and Mage, 1929].

This relationship of contracture to the evolutionary stage attained by the muscle fibre is well in accord with the facility with which smooth muscle responds to various stimuli by contractures, which in certain cases at least [Bozler, 1930 *a*, *b*] entail a notable expenditure of energy and thus cannot be interpreted simply as a manifestation of extreme muscular viscosity.

It fits in well also with the characteristic properties of the process of excitation which is the basis of contracture: slowness of development

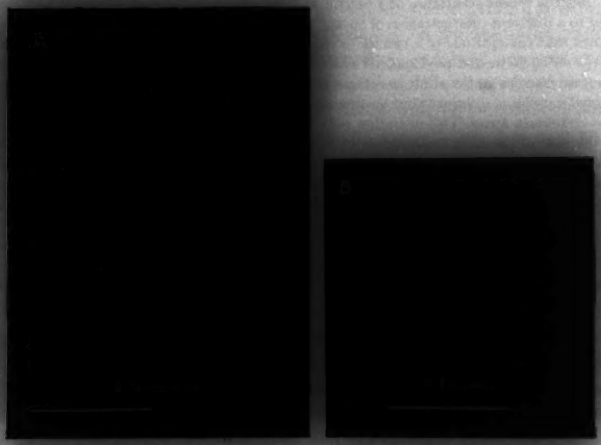

Fig. 12. A. Isotonic neuro-muscular tetanus (5 g. load) of a brown frog (*R. temporaria*) showing strong neuro-muscular contracture. Note the normal start of the plateau of the tetanus (latent period of contracture) and the dome which the contracture then produces. B. Neuro-muscular tetanus identically registered and provoked—at the same frequence of about 60 break shocks per second—of the gastrocnemius of a green frog (*R. esculenta*) of the same weight and the same state of nutrition, at the same temperature, 18° C. Time in seconds. Reduced by $\frac{1}{4}$.

Fig. 13. Myotonic rigidity in man. Patient, 39 years, showing an accentuated myotonic myopathy. Myograms of the superficial flexors of the fingers. Registration by means of a "cardiographic" pneumograph applied over the muscle. Faradization of the motor point at a frequence of 40 break shocks per second. Note the prolonged contracture which follows the first tetanus, its disappearance after a more prolonged faradization, and its complete reappearance after a rest of 5 min. Time in seconds. Reduced by $\frac{1}{4}$.

and dissipation, absence of propagation along the fibres, polar localization (cathodal), and absence or feeble development of the phenomenon of adaptation. These characteristics of the process of excitation in contracture present an analogy, already noticed by Biedermann [1895], with those of the visible reactions to galvanic stimuli by the undifferentiated protoplasm of numerous Protozoa.

Reversible contractures of the skeletal musculature of vertebrates which might be provoked by electrical, alkaloidal, or nervous stimuli, seem thus to be a primitive form of reaction of the muscle fibre. Tendency to contracture is the expression of a vestigial property, seemingly without any functional rôle, and of variable intensity according to the evolutionary stage of the muscle, its state of nutrition, and its functional specialization.

Summary.

1. The gastrocnemius of *R. temporaria* presents a particular tendency to contracture by direct or indirect electrical and alkaloidal stimuli. The better the state of nutrition of the muscle, the stronger is this tendency.

2. The chronaxie of contracture of the gastrocnemius of *R. temporaria*, measured by means of metallic electrodes and "longitudinal" excitation, is about a hundred times that of ordinary contraction.

3. Very brief direct electrical stimuli (for example break shocks), of physiological intensity, although maximal as far as the twitch is concerned, do not cause contractures of the curarized gastrocnemius when applied singly, but become effective only when repeated often enough and at an adequate interval, because of summation of stimuli. Two successive stimuli can suffice. In order to be effective at the ordinary temperature the interval ought to be between $\pm 2\sigma$ and $\pm 8\sigma$, with an optimum of $\pm 4\sigma$.

4. A single stimulus is equally ineffective in the case of indirect stimulation of the gastrocnemius of *R. temporaria*. In order to provoke a contracture of this muscle it is necessary to obtain a summation of nerve impulses. Neuro-muscular contraction provoked by two stimuli of adequate interval ($1{\cdot}5$–8σ, with an optimum of $\pm 4\sigma$) presents complete myographic analogy with the contracture elicited by summation of two direct stimuli on the curarized muscle.

5. Neuro-muscular contracture is selectively sensitive to (*a*) fatigue, (*b*) heat, (*c*) atropine and related alkaloids. The "curarization" of neuro-muscular contracture by atropine may be explained by the particular toxicity of this alkaloid for substances of long chronaxie.

6. The monophasic electromyogram of neuro-muscular contracture consists in a non-oscillatory negativity of the active electrode. The potential developed, which is very feeble, is proportional to the tension developed by the contracture. Its appearance seems to precede on the electromyogram the first manifestation of tension of the contracture.

7. Myotonic rigidity of human pathology presents striking analogies in properties and mechanism with neuro-muscular contracture in Anura.

8. Contracture by excitation of skeletal muscle seems to be a primitive form of reaction of the muscle fibre, connected with an "excitable substance" of long chronaxie, and characterized by the slowness of development and dissipation of its excitatory process, its absence of wave-like propagation, and its persistence throughout the duration of a galvanic stimulus (absence of adaptation).

9. A comparative study of the same muscle, such as the gastrocnemius, in various Anura, suggests that the tendency of a muscle to contracture depends on its functional specialization, in the sense that habitual participation of the muscle in a rapid alternating movement (swimming and jumping) accelerates its evolution towards a stage characterized by no, or only a feeble, aptitude for contracture by excitation (confirmation of the hypothesis of Freund and Rückert).

I wish to express my sincere thanks to Prof. Sherrington for undertaking the translation of this paper and to Dr H. E. Hoff who assisted in this task.

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EXPLANATION OF PLATES.

Plate I.

Isometric myogram and electromyograms of the gastrocnemius of a frog showing no neuro-muscular contracture. Decerebrate animal. Tendon-muscle leads; proximal negativity (muscular) indicated by a downward displacement of the string. String tension: 50 mm. per millivolt; sensitivity reduced to 5 mm. per millivolt after the muscle was connected. From top to bottom; time in seconds, electromyogram, myogram. Temp. 20° C. Reduced by $\frac{1}{3}$. A. Single maximal twitch (indirect excitation). B and C. Double tetanic contractions elicited by two break shocks at intervals of $3{\cdot}5\sigma$ and 10σ respectively. D. Passive variations in tension of the same muscle.

N.B. On these records, as on those of Pls. II and III, the large initial phase of the monophasic electrogram is not seen, because of the amplitude and very great rapidity of movement of the string. One can only see the positive deflection which succeeds it. The small regular oscillations in the string record are due to induction of the alternating current of the building on the very loose string.

Plates II and III.

Isometric myogram and electromyogram of the gastrocnemius of two brown frogs showing a strong neuro-muscular contracture. Same experimental conditions as for Fig. 1. Tension of the string: 40 mm. per millivolt; sensitivity reduced to 3 mm. per millivolt after the preparation was connected. Reduced by $\frac{1}{3}$. A and D. Double tetanic contractions elicited by two break shocks of just supramaximal intensity applied to the sciatic at the optimum interval of $3{\cdot}5\sigma$. Neuro-muscular contracture. B. Double tetanic contraction provoked by two stimuli at an interval of 10σ. Absence of contracture. C. Single maximal twitch. No contracture.

N.B. Contractions A, B, C, and D, were elicited in rapid succession, in this order. Explanation in text.

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EXCITABILITY OF THE SINGLE FIBRE NERVE-MUSCLE COMPLEX.

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Introduction.

The relation between the intensity of an electrical stimulus and the minimum duration for which it must be applied in order to be effective has been studied by numerous observers who have used many different kinds of muscle and various nerve-muscle preparations. The present work deals with this relationship between duration and strength of an electrical stimulus in the single fibre nerve-muscle complex. The preparation which has been used for this purpose is the retrolingual membrane in the tongue of the frog, *R. pipiens*. Data have been obtained from indirect stimulation of nerve fibres lying in the membrane as well as from direct stimulation of both normal and curarized muscle fibres. It has often been possible to make all these measurements while observing throughout the response of one and the same muscle fibre. In this respect work with the retrolingual preparation has a great advantage over the single fibre measurements of Jinnaka and Azuma [1922] and of Watts [1924], using the whole sartorius muscle, stimulated by a pore electrode.

Purpose.

The general significance of the strength-duration relationship lies in the theoretical conclusions that have been drawn from the experimental results. Since these results have been, in the main, conflicting, it is but natural that several opposing interpretations have been given to the data. The purpose of the present experiments has been to secure a direct test of the merits of two of these interpretations using a system which as far as possible avoids the sources of error that have been responsible for the conflict in previous results.

[1] Fellow in Biology, National Research Council.

There are two leading schools of opinion with respect to the interpretation of the strength-duration curves, one of which is that headed by Lapicque [1926]. Working on many kinds of nerve-muscle preparation, this observer finds that all strength-duration curves are identical if they are expressed in terms of certain units. According to his terminology the unit of intensity is the "rheobase," while the "chronaxie" is taken as the unit of time. Lapicque has recently produced [1931 *a*] a "canon" for the strength-duration curve scaled in these units.

Furthermore, the chronaxie plays an important rôle in the theory of isochronism which Lapicque has put forward. According to this hypothesis, the transmission of excitation from a nerve to its muscle is possible only when the chronaxies of both are approximately equal. Conversely, he believes that the blocking of such transmission is due to a condition of "heterochronism" which occurs when the two chronaxies differ by more than a ratio of 1 : 2. In the case of a curari block, heterochronism is supposedly produced by an increase in the chronaxie of muscle, that of nerve remaining unchanged.

Results in apparent conflict with this "law of isochronism" were obtained by Lucas [1907], and these have been extended lately by Rushton [1930, 1931, 1932 *a*]. Both observers used experimental conditions which are different from those of Lapicque. They find that the strength-duration curve is usually compound and can be separated into at least two components. One of these has a very long chronaxie[1] and is called the "α-curve." The second, or "γ-curve," has a chronaxie of the order of that found on stimulation of the nerve trunk. Both Lucas and Rushton have presented evidence which indicates that the γ-curve represents the excitability of nerve, while the α-curve measures the excitability of the muscle fibres. They find, for example, that curari abolishes the γ-curve while the α-curve is unchanged. This view, therefore, denies the importance or necessity of isochronism in the normally functioning complex and generally presumes that the block in transmission produced by curari is due to an effect upon the myoneural junction.

It has already been said that these two opposing views, of Lapicque on the one hand and of Lucas and Rushton on the other, derive from conflicting experimental results. Whence does this conflict arise? There

[1] Rushton [1932] has suggested that the chronaxie of these curves, whether "canonical" or not, should be called by Lucas' term "excitation time," since Lapicque [1931 *a*] denies that it represents "true" chronaxie.

have been numerous explanations. Jinnaka and Azuma [1922] have suggested that the discontinuities found by Lucas in the strength-duration curve represent changes in the number of excitable elements responding. Davis [1923], on the other hand, found that the chronaxie of muscle increased with the size of stimulating electrode. He was confirmed by Watts [1924]. In most of their work Lucas and Rushton have used large fluid or block electrodes, while Lapicque has employed chlorinated silver needle electrodes. The compound curves may therefore be ascribed to the effect of electrode size on the strength-duration curve of muscle. Rushton [1932 *d*] subscribes to the validity of this explanation, and points out that the use of large electrodes makes it possible to bring out more clearly the difference between the excitabilities of nerve and muscle in the gross preparation. Lapicque at first [1926] discounted the results of Lucas as due to errors. In his latest papers (1931 *a*, *b*), however, he has withdrawn from this position and ascribes the results to the experimental conditions, at the same time concluding that the α-curves are "uncanonical" and do not measure the "true" excitability. Still a fourth explanation has been presented in a paper by Moore and Brücke [1931] which appeared after the present work had been completed. It will be taken up in a later section.

Now one of these explanations—that of Jinnaka and Azuma—holds that these conflicting results are due to an error introduced by measurements on a large population of excitable elements. This explanation is not without a basis of corroborating evidence from other observers. For example, Lapicque [1926] finds that the chronaxies of different nerve fibres vary inversely with the fibre diameter. Both my own experiments and those of Moore and Brücke [1931] show that individual muscle fibres may have widely different chronaxies. It therefore is quite legitimate to ask just what is represented by the averaged strength-duration curves obtained from the gross preparation.

This difficulty is obviated by the use of a single fibre preparation. Jinnaka and Azuma [1922], Davis [1923], and Watts [1924] have reported measurements of the strength-duration curves of single muscle fibres in the sartorius of amphibians. These observers have confined themselves, in testing Lapicque's theory, to a study of the effect of curari on the chronaxie of the muscle fibre. Jinnaka and Azuma, on the one hand, find that the chronaxie is increased by curarization in accordance with Lapicque's views. On the other hand, Watts finds no such change. This disagreement is possibly due to the fact that in neither group of experiments were the measurements carried out on the identical fibre

both before and after curarization. In view of the difference in the chronaxies of individual fibres either result is equally probable, and does not furnish an adequate test of the theory of isochronism.

The present work has been carried out on a preparation in which the chronaxies of one and the same fibre can be determined both before and after curarization. Furthermore, a more complete test regarding the merits of the different theories of excitation can be carried out since it is also possible to stimulate nerve fibres lying in the retrolingual membrane and thus obtain the chronaxies of both components of the nerve-muscle complex. The response used has always been "all-or-none" under the conditions of the experiments [Pratt, 1930; Gelfan and Gerard, 1930], and therefore a sharp index of response is available. Furthermore, it is possible to distinguish directly between a response due to nerve stimulation and a response caused by direct excitation of the muscle fibre. This distinction is possible because indirect stimulation through the nerve causes a contraction of the entire motor unit, while on direct stimulation only one fibre responds. The picture seen through the microscope is therefore quite different in the two cases. The distinction does not hold in the gross preparation, where a more involved analysis is employed [Rushton, 1932 *c*], in order to distinguish between responses due to direct and indirect stimulation.

Preparation.

The retrolingual membrane of the frog's tongue, a preparation which was first described by Thoma [1873], has lately been recalled to physiologists by Fischl and Kahn [1928], Gelfan [1930] and by Pratt [1930]. The single muscle fibres are widely separated from each other so that they can be stimulated individually. It is also possible, in many cases, to stimulate them through the nerve fibres lying in the membrane. Under ordinary conditions the preparations can last eight hours or more, and numerous experiments can be performed on any one particular fibre. Moore and Brücke [1931] have already reported chronaxiemetric results on this preparation. The present measurements were completed in the main before their data were published, and are obtained by the use of a different technique so that the results are therefore somewhat different. By using a modification of Moore and Brücke's technique I have been able to confirm their measurements and show the underlying cause of the differences in our results. I cannot, however, agree with their conclusions, which will be discussed in detail later.

METHOD.

The method of preparation employed is a modification of that described by Pratt and Reid [1930] which permits the use of trans-illuminated membranes with intact circulation. A longitudinal superficial incision is made in the ventral surface of the everted tongue of the decerebrate frog. The various layers of tissue are carefully cut along the mid-line without injuring the larger blood vessels. This tissue is then pinned back to expose the retrolingual membrane. The preparation is covered with Ringer's solution (modification of Stella, described by Parkinson, 1930, p. 226). A comparison of excised and intact mem-

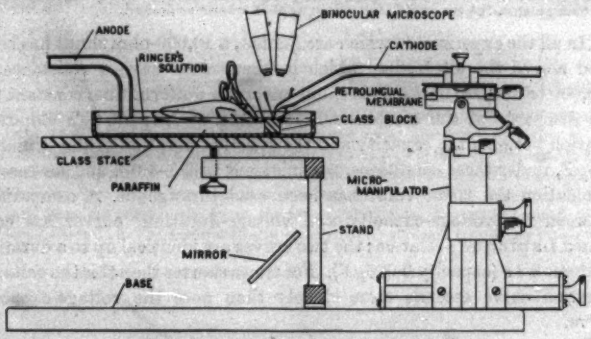

Fig. 1. Diagram of the experimental arrangement.

branes has convinced me that the latter is greatly superior, offering as it does a large number of fibres which are uninjured and therefore capable of great steadiness in threshold. Moore and Brücke [1931] make the statement that the intact preparation does not permit clear observation of the muscle fibres. In the course of my work, however, I have demonstrated the muscle fibres to many people, most of whom have had little or no training in microscopic observation and in no case has there been any difficulty in observing a response by a single muscle fibre.

Stimulation has, in general, been by means of condenser discharges, essentially as described by Lapicque. The stimuli are applied through a localized cathode which consists of a large-bore glass tube (5–6 mm. inside diameter) pulled out in a micro-flame to a capillary having an opening about 20–60 microns in diameter. This tube is filled with Ringer's

solution and is connected to a Zn—$ZnSO_4$ electrode by means of an agar Ringer's bridge. The anode is diffuse and is also made of agar Ringer's and Zn—$ZnSO_4$. The relatively large opening of the cathode assures all-or-nothing responses [Pratt, 1930; Gelfan and Gerard, 1930], while the wide spacing between the fibres of the membrane permits stimulation of only one fibre.

The experimental arrangement is easily grasped from a study of Fig. 1. The frog is placed in a petrie dish which is cemented to a movable glass stage. To the bottom of the dish is cemented a polished glass block 1 cm. square. The tongue is everted and the cut tissues are pulled out so that the retrolingual membrane lies flat on the block. The membrane is illuminated from below with the aid of a mirror and observations are made with a binocular microscope (magnification 70×). A layer of paraffin whose level is slightly below the top of the glass block covers the petrie dish to permit pinning the tissues. The anode dips into the Ringer's solution which covers the preparation, the cathode being held over the membrane in a micro-manipulator. By means of the various adjustments on the manipulator, the tip of the electrode is applied wherever desired.

In all the experiments which are quoted, a 10,000-ohm shunt has been used across the electrodes, which in themselves have a resistance of 150,000 to 250,000 ohms. This shunt ensures a uniform time constant for the discharge of the condensers. According to Lapicque's empirical relation between the capacity and the effective duration of the stimulus, 1·0 μF, under these conditions, is equivalent to 3·7–4·0σ, and for ease in calculation the latter value has been used throughout. A comparison between the voltage-capacity and voltage-duration[1] curves has confirmed Lapicque's relation: the two curves are identical up to a duration of about 0·1σ (capacity 0·025μF). For times shorter than this the voltage-duration curve ascends more steeply than does the voltage-capacity curve.

Results.

The experimental results can be divided into two main sections. The first group deals with a comparison of the voltage-capacity curves for direct and indirect stimulation. The second group is concerned with the effect of curari upon the voltage-capacity curve of a single muscle fibre. Several kinds of subsidiary experiments will be dealt with after the results of the first two groups are described.

(a) *Direct and indirect stimulation of a single muscle fibre.*

In twenty-four preparations it has been possible to stimulate both nerve and muscle fibres. The results show definitely that the chronaxie is much smaller for the nerve fibres than it is for the muscle fibres.

[1] The latter have been obtained with a timing device which has been newly designed by Mr A. J. Rawson, of the Johnson Foundation. It will be described by him elsewhere.

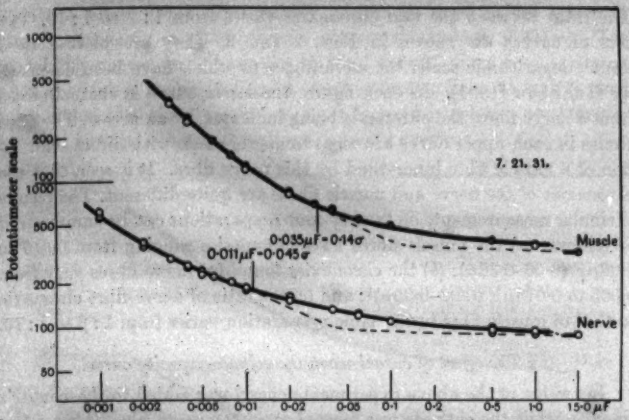

Fig. 2. Voltage capacity curves plotted in logarithmic coordinates. Ordinates: voltage in terms of divisions on a 1000-unit potentiometer scale. Abscissae: capacities in μF. The lower curve is for a single nerve fibre. The upper curve is for one muscle fibre innervated by this nerve fibre. The filled circles are measurements before curarization, while the clear circles represent measurements after curarization. The chronaxies of each tissue are given in the figure, the ratio of chronaxies of nerve and muscle fibres being about 1:3. The curve drawn in broken lines represents Lapicque's "canon."

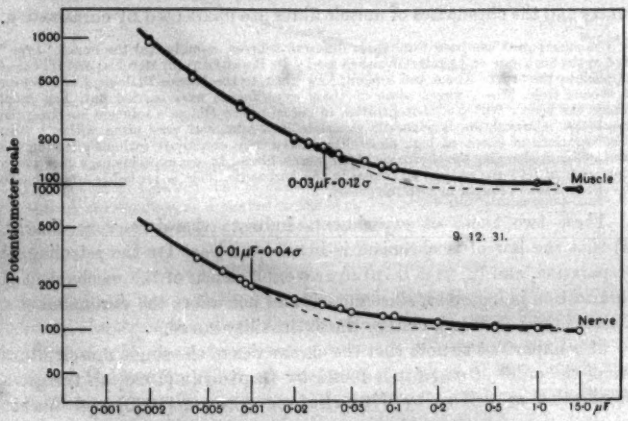

Fig. 3. Another set of curves from a different preparation.

The ratio between the two chronaxies varies from 1 : 2 to 1 : 10. Two sets of curves are shown in Figs. 2 and 3. They are plotted on a double logarithmic scale, the advantages of which have been discussed by Rushton [1931]. In each figure, the lower curve is that obtained from a nerve fibre, the chronaxie being indicated by an arrow. The filled circles in each upper curve are measurements made with direct stimulation of a muscle fibre innervated by this nerve fibre. It is seen that the chronaxies of the nerve and muscle fibres are quite different. The results of similar measurements on twenty-four preparations can be summarized as follows: (*a*) the muscle fibres have chronaxies ranging from 0·016 to 0·08μF (0·06–0·32σ); (*b*) the chronaxies found for nerve fibres vary from 0·005 to 0·013μF (0·02–0·05σ)[1]; and (*c*) the ratio of nerve-fibre chronaxie to that of muscle fibre in the same preparation varies from 1 : 2 to 1 : 10.

(*b*) *The effect of curari upon the voltage-capacity curve.*

In twelve of the above experiments curari was added to the Ringer's solution while the cathode was located upon the nerve fibre. After a few minutes, a block in transmission was established. The cathode was then replaced upon a muscle fibre whose uncurarized excitability had been previously measured. Its excitability after curarization was then determined. Figs. 2 and 3 present the data for such measurements as well. The experimental values after curarization are indicated as open circles in the upper curves. It is clear from these figures that the excitation curves and the chronaxies of muscle fibres are unaffected by curarization.

The curari used has been from three different sources, namely: (*a*) the curari "type" used at the Sorbonne and kindly furnished me by Dr Rushton; (*b*) Merck's; and (*c*) curari supplied by the firm of Eimer and Amend, New York, to the Marine Biological Laboratory at Woods Hole, Mass., where some of these experiments were carried out. The usual dosage has been a 0·01 p.c. concentration of curari in the Ringer's solution covering the membrane. Curarization is generally complete in 3 min. and very often within 1 min. Concentrations of curari as high as 0·05 p.c. have been employed without affecting the excitability or changing the chronaxie of the muscle fibres. In one experiment, a curari concentration of 0·07 p.c. caused a *decrease* in the chronaxie. This is probably ascribable to the effect of salts brought in with the curari.

These two types of experiments indicate therefore very clearly, (1) that the law of isochronism is invalid, at least for the retrolingual preparation, and (2) that Lapicque's explanation of the mechanism of curarization is incorrect, since curari does not affect the chronaxie of a muscle fibre nor does it change the excitability curve.

It is important to note that the chronaxies of the single muscle fibres are of the order of magnitude found by Lapicque [1926] on the gross preparation as well as by Jinnaka and Azuma [1922] and Watts

[1] The surprisingly small chronaxies of these nerve fibres will be discussed later.

[1924] on single fibres. Presumably, therefore, these values represent "true" chronaxie in Lapicque's newer terminology [1931 *a*]. The excitability curves do not agree with the "canon" given by Lapicque [1931 *a*] which is shown in Figs. 2 and 3 in broken lines, though it must be admitted that this "canon" was put forward in relation to strength-duration curves, and not to voltage-capacity curves.

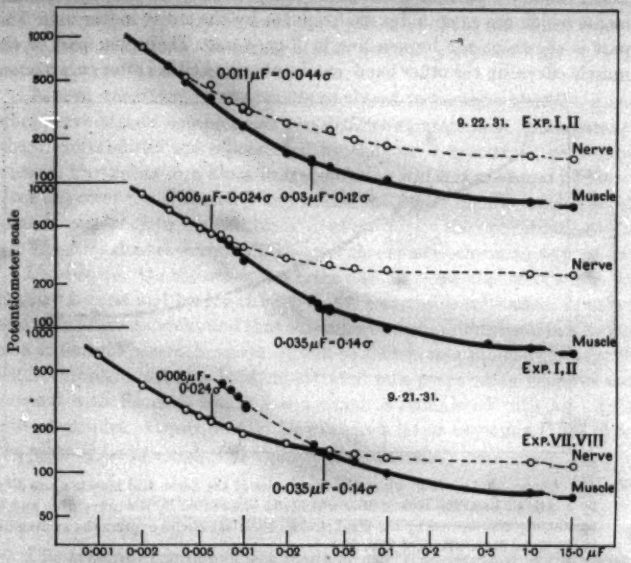

Fig. 4 *a*. Compound curves similar to those described by Lucas, but obtained from the single fibre preparation. The suppressed branches of both the nerve and muscle-fibre curves are obtained as described in the text. The lower and middle compounds are from the same fibres. The upper is from another preparation.

(c) *The production of compound curves.*

A striking, but entirely to be expected, illustration of the difference between the strength-capacity curves of muscle and nerve fibres is shown in Figs. 4 *a*, *b*. Such compound curves are obtained in the following manner. The localized electrode is applied at a place where a muscle fibre and a nerve fibre are contiguous. Measurements are then made stimulating the muscle fibre.

It is perfectly clear from Figs. 2 and 3 that the muscle curve ascends at a faster rate than does the curve for nerve. Eventually therefore a capacity is reached for which the threshold of the nerve is below that of the muscle. The response then changes in the manner already described, there is a discontinuity in the voltage-capacity curve, and measurements are now being made along a curve representing nerve excitation. Furthermore, the lower portion of the nerve curve can be obtained by measurements which use as an index the response by the entire motor unit. This part is shown as the broken line in Figs. 4 *a*, *b*. The upper part of the muscle curve, on the other hand, can be obtained either after curarization

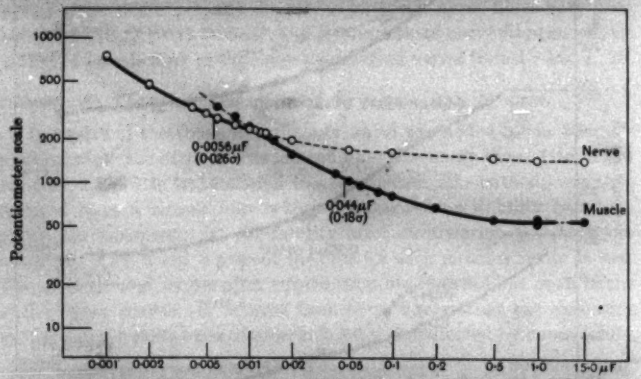

Fig. 4 *b*. A compound curve in which the chronaxies of the nerve and muscle fibres differ by a ratio of 1 : 8. The muscle fibre which was innervated by the nerve fibre had an excitability represented by the filled circles. The clear circles express the excitability of the nerve fibre. (Exp. of 3. v. 32.)

or, more simply, by moving the electrode away from the vicinity of the nerve fibre. The broken line of the muscle curve in Fig. 4 *a* (lower set) and Fig. 4 *b* represents this portion.

These compound curves, except for the different time relations, are strikingly reminiscent of the curves obtained by Lucas [1907] and by Rushton [1930, 1931]. For the moment, we shall leave open the question as to the relation which the compound curves obtained from the gross preparation bear to those found in the study of single fibres. The point which I wish to emphasize now is that in the present case, it is demonstrated directly that the composite nature of the curves in Figs. 4 *a*, *b* is due to the excitation of two different elements, namely, the muscle fibre and the nerve fibre.

The curves shown in Fig. 4 *a* also illustrate how different kinds of compounds can be obtained. The lower and middle sets are the identical fibres, but the electrode has been moved slightly so that the relative thresholds of the nerve and muscle are different. The curves therefore cross at different values of the capacity. A second way of changing the crossing point is shown in the upper set. Although the relative thresholds are intermediate to those in the other curves the crossing point comes at a much smaller capacity because the chronaxies of the nerve and muscle fibres, and therefore the forms of the strength-duration curves, more nearly approach each other.

In sum, therefore, all the results produced so far show that the single fibre nerve-muscle complex has two distinct excitabilities. Structurally, these excitabilities are represented by the two components, nerve and muscle. Furthermore, a block in transmission which is produced by curari does not alter the chronaxie, and unless the curari dose is much greater than is necessary to cause a block does not affect the excitability curve.

These results are in conflict with the theory of isochronism expounded by Lapicque. On the other hand, they are in agreement with the view, held by Lucas and by Rushton, that the excitable substances of nerve and muscle are different and that this difference manifests itself in a condition of normal heterochronism. It will be shown, in a moment, that such heterochronism can also be demonstrated in a preparation which is not covered with Ringer's solution and which is stimulated with Ag—AgCl wire electrodes. Consequently, Lapicque's latest objection [1931 *b*] to the view of Lucas and of Rushton disappears.

(*d*) *Heterochronism in preparations not covered with Ringer's solution.*

This type of experiment was made after the appearance of the paper by Moore and Brücke [1931], already mentioned. These authors use large Ag—AgCl wire electrodes for stimulating single muscle fibres in the retrolingual membrane. They also stimulate the hypoglossal nerve trunk while observing, in some cases, the response of muscle fibres. The chronaxies which Moore and Brücke obtain are much larger than those given above. Thus, they find that the chronaxies of the nerve trunk are 0·9–1·5σ, while for the muscle fibres the chronaxie varies from 2·5 to 70σ. Moore and Brücke also report and illustrate remarkable differences between the response to direct and indirect stimulation. With the relatively large capillary electrode, I have been unable to find any such peculiarities as partial contracture and slow relaxation upon direct stimulation which are reported by these observers.

For these several reasons I have repeated their experiments, using, however, the intact preparation. This has been mounted in the chamber shown in Fig. 1, the floor of which is covered with a layer of moist filter paper (cut out to clear the glass block). To the filter paper is attached a chlorinated silver-wire anode running the length of the membrane underneath it and perpendicular to the muscle fibres. Since the glass block and therefore the central portion of the membrane is higher than the filter paper, the preparation is not covered by Ringer's solution. For a cathode I have used (*a*) either another chlorinated silver wire running parallel to

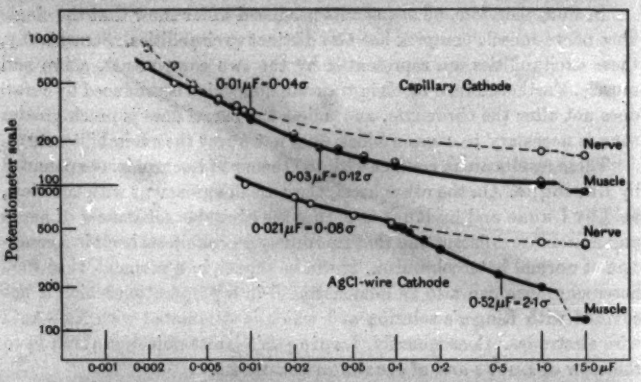

Fig. 5. Compound curves obtained from the identical fibres, with electrodes of different sizes. The lower compound has been obtained by using a silver-wire cathode, the upper with a capillary electrode. The broken circles in the upper muscle curve represent measurements after curarization.

the anode, (*b*) a silver wire bent downwards in a right angle at the tip so that only one fibre might be stimulated, or (*c*) the usual capillary electrode.

I have been able to confirm the unusual appearance of the response to direct stimulation in the case where the cathode is parallel to the anode (type (*a*)). A somewhat similar result is also obtained when two wire point electrodes are used. Responses of this type are presumably similar to the graded contractions of Fischl and Kahn [1928], Gelfan [1930], and others. However, with a cathode of type (*b*), the responses are exactly like those obtained with the capillary electrode. I am unable to explain these differences in response with the different electrode systems.

The results of measurements with cathode (*b*) and (*c*) are very interesting. They are shown in Fig. 5. The lower set of curves is obtained with a chlorinated silver-point cathode, both for a nerve fibre and for a muscle fibre. The upper set is from subsequent measurements on the same elements using a capillary cathode. The chronaxie of the muscle fibres stimulated with the wire electrode is about $2{\cdot}1\sigma$ ($0{\cdot}52\mu$F). That of the nerve is about $0{\cdot}1\sigma$ ($0{\cdot}025\mu$F). With the capillary cathode the chronaxies of the same nerve and muscle fibres are about $0{\cdot}12\sigma$ ($0{\cdot}03\mu$F) for the muscle fibre and $0{\cdot}04\sigma$ ($0{\cdot}01\mu$F) for the nerve.

This result shows quite clearly why the values of the chronaxies quoted by Moore and Brücke are higher than those obtained with the capillary electrode. The increase is due to the larger size of the electrodes which they employ. The effect of electrode size upon the chronaxie of muscle is already well known from the data of Davis [1923] and of Watts [1924]. The present work, however, is the first to show that the chronaxie of nerve is affected in a similar manner. The amount of increase is smaller for nerve than it is for muscle, but nevertheless is appreciable. The ratio of the chronaxies with the two electrode systems is 1 : 2·5 for the nerve fibre and about 1 : 20 for the muscle fibre.

TABLE I. Effect of raising the electrode above the muscle fibre.

Threshold	Chronaxie (μF)	Position of electrode
130	0·065 0·063	Touching membrane
310	0·082 0·083	Raised
480	0·14 0·13	Raised
500	0·13	Raised
200	0·085	Lowered
145	0·077	Lowered
130	0·078 0·077 0·074	Lowered
100	0·071 0·070	Lowered

The results of a somewhat similar experiment are shown in Table I. In these measurements the capillary electrode has been placed at different heights above the membrane and the chronaxie has been determined at each position of the electrode. As the electrode is raised above the membrane the area of the muscle fibre over which current is distributed becomes greater. In this respect raising the electrode is identical with an increase in the size of stimulating electrode. Column 2 of

Table I shows that the chronaxie increases when the electrode is raised and decreases when it is again lowered.

An adequate treatment of the physics of current distribution from a point electrode is beyond the scope of this work. It seems probable, however, that the change of chronaxie with electrode size can be accounted for by the resultant change in configuration of current distribution.

The results just presented have a bearing upon the compound curves of Lucas and of Rushton and their relation to the composite curves of Figs. 4 and 5. Before discussing this subject, however, two further points are of interest. Moore and Brücke [1931] conclude, from their results, that the heterochronism which they found in the retrolingual membrane complex is not contradictory to Lapicque's theory. This conclusion is based, in part, upon an argument from analogy. It has already been stated that the chronaxie of a nerve fibre varies inversely with the fibre diameter [Lapicque, 1926]. Moore and Brücke carry this datum over and apply it to muscle fibres. From this analogy they reason that heterochronism in the retrolingual membrane is due to the fact that the muscle fibres, in their experiments, are stimulated at the finely branched tips, where the chronaxie is presumed to be very large, while the nerve fibres are stimulated in the nerve trunk and therefore at their full diameter.

Moore and Brücke further apply this argument to an explanation of the curves of Lucas and Rushton. Rushton [1932*c*] has answered this explanation from a standpoint which assumes that Moore and Brücke have demonstrated the dependence of chronaxie upon the diameter of the muscle fibre in the region of the cathode. Let us examine, however, the data which Moore and Brücke produce. In stimulating the muscle fibres of the retrolingual membrane, these authors use two parallel silver wires. The cathode lies clear of the fibres and near their terminations. The anode is placed upon the tongue tissue. Stimulation is, therefore, always accomplished at the tips of the fibres. Now, Moore and Brücke find that the fibres having the finest terminal branches exhibit a larger chronaxie than do the fibres ending in blunt processes. This is the extent of their proof for the assumption that the chronaxie varies inversely with the diameter of the fibre at the locus of stimulation. Using both the pointed wire and the capillary electrodes as cathode I have searched along single muscle fibres to discover whether this procedure, which is experimentally better suited to the purpose, produces a change in the chronaxie of the muscle fibre. In all the measurements which have been made, there is no change in the chronaxie until the very tips of the fibres are reached, although there is usually a tapering off in

the fibre diameter. Results of stimulation at the tip can be divided into three groups. In one group of fibres, no change in the chronaxie is observable. In a second group, which is numerically the largest, it has not been possible to produce any response by excitation at the tip even though the stimuli are increased a hundredfold above the rheobase for stimulation in the body of the fibre. In a third group of muscle fibres the response to stimulation at the tip shows the curious long latent period reported by Moore and Brücke. This latent period is of the order of seconds and may well be due, as the above authors (following Hintner [1930]) believe, to a decrement in transmission of excitation. The threshold for this response is very irregular, changing as much as 50 p.c. Because of this, it is impossible to be certain of the chronaxie measurements. However, the chronaxie apparently increases, sometimes double or more.

TABLE II. Variation of chronaxie in different muscle fibres when locus of stimulation is changed.

Fibre	Locus of cathode	Threshold potentiometer scale	Chronaxie μF	Remarks
I	Body of fibre	140 ($V=10$)	0·062–0·065	Small fibre
	Tip of fibre	185	0·058–0·062	
II	Body	122 ($V=6·5$)	0·068–0·069	Fibre diameter about 3 times that of I
	Tip	($V=45$)		
		(No response at full strength)		
III	Body	130 ($V=10$)	0·06–0·062	Large fibre
	Tip	370 ($V=40$)	0·15	Latent period of about 2 sec.

In Table II are shown sample results of measurements from each of these three different groups, made on three fibres from one preparation. No change in chronaxie was observed in the first fibre. The second fibre could not be stimulated at the tip, while there is an increase in the chronaxie of the third fibre from 0·06μF (0·24σ) to 0·15μF (0·6σ), accompanied by a latent period of approximately 2 sec.

These results show therefore that Moore and Brücke's assumption, that the chronaxie of a single fibre depends upon the diameter of the fibre at the locus of stimulation, may be valid for some muscle fibres. However, this assumption apparently cannot be used to explain the heterochronism found in the gross preparation, since the extremely long latent period exhibited in the response of these fibres to stimulation at the tip is not to be found in the measurements of Rushton [1931]. It is hardly necessary to remark that the explanation offered by Moore and Brücke does not apply at all to the present work.

A second type of experiment for testing this assumption of Moore and Brücke consists in determining the chronaxies of different fibres of various diameters in the same preparation. The diameters of the fibres in the retrolingual membrane vary from about 15μ to 60μ or more. No relation has been found, however, between fibre diameter and chronaxie, such as is postulated by Moore and Brücke. On the contrary, it has been often found that large fibres may have chronaxies as much as four

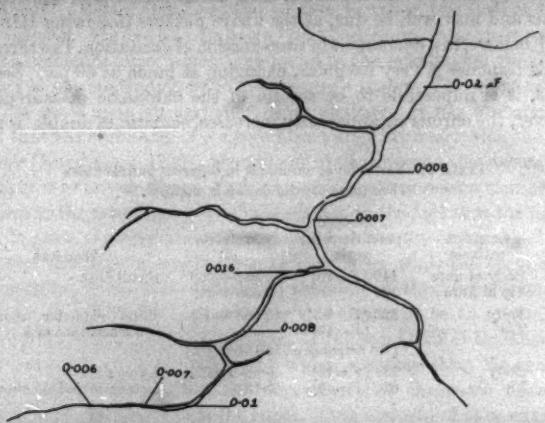

Fig. 6. Diagram of a nerve branch containing at least two fibres. This branch has been stimulated with a capillary electrode located at the various points to which reference lines have been drawn. Throughout the stimulation the response of one muscle fibre about 4–5 mm. distant was observed so that the chronaxies obtained along the branch represent those of a single nerve fibre. These values are shown in the figure and indicate that the chronaxie of a nerve fibre is not a fixed value but varies in a manner discussed in the text.

times greater than the chronaxies of very small fibres. The attempt of Moore and Brücke to reconcile their chronaxiemetric results with the theory of isochronism therefore seems to me to be without adequate experimental justification.

A further point to be discussed is in relation to the extremely small chronaxies which I have found for the nerve fibres. A possible explanation for this was pointed out to me by Prof. E. D. Adrian while he was a visitor at the Johnson Foundation. This explanation assumes that the small chronaxies are due to the fact that the single nerve fibres are being

stimulated in the membrane, where they are relatively free from connective tissue. It is in line with the results of Bishop [1928], who found that the chronaxie of a nerve trunk decreases when the sheath is removed. In agreement with this explanation I find that the chronaxie of a hypoglossal nerve fibre is approximately doubled when it is stimulated at one of the branches which is sent out by the nerve trunk into the tongue.

This change of chronaxie along the nerve fibre which seems to be related to the amount of inert connective tissue surrounding the fibre is perhaps illustrated more strikingly in the following experiment. The nerve branch which is shown diagrammatically in Fig. 6 has been stimulated at various locations indicated by reference lines in the figure. Near each line is also given the chronaxie found at this point. This nerve branch probably contained at least two fibres. However, all the measurements shown in this illustration have been obtained while observing throughout the response of one muscle fibre, and therefore represent the change in chronaxie which is found at various points along a single fibre. The muscle fibre itself had a chronaxie from 0·04μF (0·16σ) to 0·056μF (0·22σ), depending upon the proximity of the capillary electrode. It was 4–5 mm. distant from the region of the nerve stimulation. The nerve measurements were made with the electrode always as close as possible to the nerve. They show that in the thicker portion of the branch the nerve fibre had a chronaxie of 0·02μF (0·08σ), while at the very thin region the chronaxie fell to 0·006μF (0·024σ).

Discussion.

The results described in this paper show that the "law of isochronism" is not valid for the retrolingual preparation. Since there is no reason to assume that this tissue is unique, we must arrive at the conclusion that the law holds no better for any other nerve-muscle complex. They also force, however, a re-examination of the meaning and value of the concept of chronaxie. A brief review of this nature will be made in the present section.

Chronaxie, as a unit of time, seemed to Lapicque [1926] to possess many advantages. It was a quantity easily determined and formed the basis of a convenient description of the strength-duration curve. The concept of chronaxie led him to the various theoretical conclusions embodied in the law of isochronism and also to the idea that the chronaxie reflects closely such properties as velocity of propagation in nerve and in muscle.

It has become more evident, however, that the experimentally determined value of chronaxie depends on a number of factors. Davis [1923] showed that the chronaxie of muscle depends upon electrode size. His results were confirmed by Watts [1924]. In the present work this dependence upon electrode size has again been confirmed for muscle and has also been found for nerve.

Lapicque [1931 *b*] has recently suggested that the experimentally measured quantity also changes with a change in the type of surrounding medium. This seems a very reasonable suggestion and is supported by his measurements. He assumes, however, that chronaxies determined when a preparation is covered with Ringer's solution do not represent "true" chronaxie, while measurements made with a preparation which is only moistened with such a solution indicate "true" chronaxie. This classification seems to me arbitrary and one which does nothing to advance the merits of chronaxie as a theoretical concept.

Results given in this paper indicate that the chronaxie of nerve fibres, besides being determined by electrode size, is also largely influenced by the amount of connective or inert tissue around the nerve. These results present further difficulties to the concept of chronaxie, since they show very clearly that the chronaxie cannot be considered as a unit of measurement independent of experimental conditions and of structural modifications in the tissue.

An examination of its definition discloses the probable causes for these changes which the value of chronaxie undergoes. The strength-duration curve is measured in terms of the liminal voltage or current which will excite when applied for any given duration. The chronaxie, τ, is then expressed as the duration at which V, the stimulus strength, equals $2R$; where R is the rheobasic strength. However, as Lapicque points out [1926, p. 234], it is not the current strength but the current density which is the effective measure of an electrical stimulus. The current density, ρ, is some function, $f(V)$, of the strength, V. This function depends upon all the variables discussed so far, namely, the geometry of the electrode system, and the specific conductivity of the solution and of the various inert and excitable tissues which may be present. Conceivably, the area over which the current is spread upon the tissue is also a factor in determining a threshold stimulus. In such a case, the relative sizes of tissue and electrode will likewise influence the strength-duration curve. Bishop [1928] has discussed various other factors which may affect the precise form of the function $f(V)$. It can, therefore, be readily appreciated that the strength-duration curve can

change its form whenever $f(V)$ is changed, and the chronaxie, or τ, at $V = 2R$ will also assume different values. It is therefore evident that it is impossible to speak of any single and unique chronaxie for a given muscle or nerve. Consequently the chronaxie can no longer be accepted as an adequate unit in the description of strength-duration curves or as a basis for theoretical discussions regarding the nature and course of the excitation process.

We are now in a position to inquire into the possibility of bringing together more closely the results obtained in the study of the single fibre nerve-muscle complex with those of Lucas and of Rushton on the gross preparation. With the presentation of Rushton's latest analysis [1932, in press], it has become quite clear that the complex in the gross preparation is composed of two elements. These are the excitable substances in nerve and muscle. The results just presented show clearly the same distinction between the excitable substances in nerve and muscle fibres. The time relations which are found for these substances are, however, different in these two types of nerve-muscle preparations. For the muscle fibres this difference, as Rushton has already assumed, is due to the effect of electrode size found by Davis [1923]. Up to the present work, however, it has been assumed that the excitation time of nerve fibres is little affected by electrode size. Indeed, the γ-curve definitely has [Rushton, 1932*c*] an excitation time of the order of magnitude found for the nerve trunk. When single nerve fibres relatively free from connective tissue are stimulated however, it is found that the excitation time also depends on electrode size, and becomes very small with small electrodes. It is exceedingly likely, therefore, that the γ-curve of gross preparations is a counterpart of the curve obtained for single fibres, but with the time relations, owing to the use of large electrodes, altered in the manner which has been discussed in the preceding pages.

Conclusions.

The measurements on the strength-duration relationship in the single fibre nerve-muscle complex yield direct evidence that:

1. Lapicque's law of isochronism is not valid, at least for the system in the retrolingual membrane.

2. The view held by Lucas and by Rushton which assumes that different excitable substances are present in nerve and in muscle is in agreement with the results obtained from a study of the single fibre complex.

These two conclusions find experimental justification from the following lines of evidence:

(*a*) The voltage-capacity curves of muscle and nerve fibres are different in shape.

(*b*) This difference can be manifested by the production of compound curves, which can be analysed directly into the two components obtained from stimulation of nerve and of muscle.

(*c*) The chronaxie of nerve fibres is much smaller than is the chronaxie of muscle fibres.

(*d*) Curarization does not affect the chronaxie or the strength-capacity curve of muscle fibres.

The present work also yields experimental evidence that:

3. The compound curves obtained in the gross preparations are strictly homologous with those obtained for single fibres. The difference in time relations in the two experimental procedures is brought about by two factors:

(*a*) the excitation time of muscle fibres is increased with increased electrode size, and

(*b*) the same effect exists, to a lesser degree, in nerve fibres.

4. The chronaxie of nerve fibres decreases with a decrease in the connective tissue sheath. This conclusion is based on the fact that stimulation of the nerve fibre in the trunk or in a branch produces an increased chronaxie.

5. Because the chronaxies of both muscle and nerve are dependent upon the electrode size while that of nerve also varies with the amount of connective tissue sheath, it seems legitimate to question the further usefulness or significance of Lapicque's concept of "chronaxie." It no longer appears to have the feature of being a simplifying and generally applicable unit of time in the description of the strength-duration curve.

6. Finally, it is concluded that the explanation offered by Moore and Brücke [1931] for the heterochronism which has been discovered in various nerve-muscle preparations does not seem to have any experimental justification. Their explanation assumes, from analogy with the results for nerve [Lapicque, 1926], that a large chronaxie in muscle is due to stimulation of the individual muscle fibres in a locus where the fibres have a small diameter. No such effect of fibre diameter upon chronaxie has been found, except in the case where the response to direct stimulation of the tip exhibits a latent period of the order of seconds.

SUMMARY.

The present investigation is concerned with the determination of the relation between the intensity of an electrical stimulus and the minimum duration for which it must be applied in order to stimulate single nerve and muscle fibres. It was undertaken with the purpose of testing the merits of the conflicting views of Lapicque [1926] and of Lucas [1907] and Rushton [1930, 1931, 1932 *a*]. The preparation used for this purpose has been the single fibre nerve-muscle complex of the retrolingual membrane of the frog *R. pipiens*.

The conclusions reached from the results of this investigation are stated in the previous section.

I wish to thank Prof. D. W. Bronk, director of the Johnson Foundation, for his kindness and help during the course of this work. I am also greatly indebted to Dr W. A. H. Rushton for the stimulation and aid which he generously bestowed during his residence at the Johnson Foundation.

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THE EFFECT OF FATIGUE ON END-PLATE DELAY.

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Introduction.

The classical experiment to demonstrate the nature of the failure of indirect excitation in fatigue is to stimulate the nerve of a muscle-nerve preparation until the muscle ceases to give a contraction, and then to show that a response may still be evoked by direct stimulation. From this result it is argued that, since the fatigue is not due to any change in the nerve (which can be shown to be conducting normally), nor to any obvious alteration in the muscle itself, it is necessary to postulate an intermediary tissue, functionally distinct from nerve and muscle, which has the property of transforming the energy of the nerve impulse into a stimulus for the muscle, and which is particularly susceptible to fatigue.

The conception of the separateness of the junctional tissue derives considerable support from the well-known experiments of Claude Bernard on curarization and from the detailed investigation of Langley [1907], who put forward the hypothesis that the intermediary is specially sensitive to such influences as nicotine and curare. Further evidence was supplied by Keith Lucas [1907] from experiments on the time relations of excitability of frogs' sartorius muscles. Lucas found that, if the point of stimulation is in the region of the muscle where the nerve enters, the strength-duration curve generally exhibited one or two breaks which he explained as being the result of stimulating at a point where the excitatory process could influence both nerve and muscle: in that part of the muscle where no nerve endings could be seen, a smooth curve was always obtained. The slowest curve he regarded as being derived from excitation of the muscle; the middle one, which was abolished by curare, he attributed to nerve; and the fastest of all, which was not affected by small doses of curare, he tentatively suggested might be regarded as

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characteristic of the neuro-muscular junction. This interpretation has not, however, been generally accepted.

It has been known for a long time that, in the indirect stimulation of muscle, the time which elapses between the moment of stimulating the nerve and the appearance of the muscle-action potential is longer than can be accounted for by conduction in the nerve, unless, as Lapicque and others have suggested, the rate of conduction in the terminal branches is slower than in the nerve trunk where it can be measured. As these branches are generally smaller than the fibres in the trunk, it is quite probable that they conduct more slowly, though this does not necessarily follow, since the experiments of Gasser and Erlanger [1926], which might seem to suggest this conclusion, were concerned with the rates of conduction in fibres of different function.

This delay in the transmission of excitation from nerve to muscle has been investigated by Forbes, Ray and Griffith [1923]. They found that if two stimuli are sent in to a motor nerve with only about 4σ between them, the second stimulus sets up an impulse which is delayed a relatively long time at the myoneural junction. The interpretation of these results which is given by Forbes, Ray and Griffith is that, at the junction of the nerve with the muscle, the excitatory condition due to the second stimulus may persist until the threshold of the muscle, raised by the first response, has fallen sufficiently to allow the second impulse to take effect. This explanation involves the assumption that the effective portion of the nerve impulse lasts as long as 5σ, a duration considerably greater than that usually accepted for frog's nerve at 20° C. Alternatively, the delay may be attributed to a separate substance which is able to remain in the excitatory state for a longer period than nerve. A means of avoiding both these assumptions will be suggested later.

The delay observed even in the transmission of a single impulse was attributed to a separate substance by Wieser [1915], who investigated the effect upon it of fatigue. Wieser's measurements were of the latent period between the stimulation of a motor nerve and the appearance of the muscle-action potential. He found that after a nerve-muscle preparation had been fatigued by tetanization, the latent period was often increased by as much as 100 p.c. Samojlov [1925] improved upon Wieser's technique by placing one lead of a string galvanometer on the nerve of a sciatic-gastrocnemius preparation at the point where it enters the muscle, and the other lead on the tendon of the muscle. In this way he avoided the possibility of error due to changes in the rate

of conduction in the nerve. He measured the effect of changes of temperature upon the delay, and from his results calculated the very high Q_{10} of 2·0 to 2·6, a value much greater than that obtained by Fulton, in whose book [Fulton, 1926] the whole subject is treated in detail. Samojlov deduced from his figures that transmission in the motor end-plate is "more chemical" than in either nerve or muscle. The temperature coefficients of biological systems cannot, however, be considered as affording very exact evidence as to the nature of those systems.

Method.

The purpose for which this work was undertaken was to discover if curare had any effect on the delay as measured by Samojlov. The

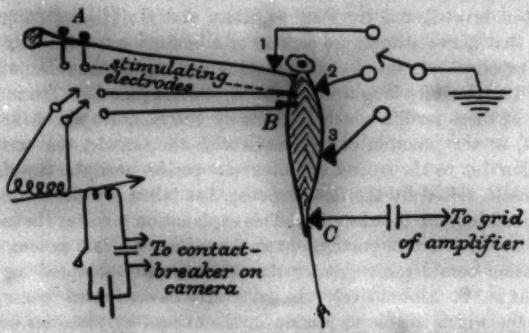

Fig. 1. Arrangement of electrodes on sciatic-gastrocnemius preparation.

arrangement of electrodes was as in Fig. 1. Since the string galvanometer cannot give accurate records of currents of short duration, a thermionic amplifier and Matthews' oscillograph were substituted. These were worked with a specially constructed rotating mirror camera of the type described by Matthews [1929]. With this it was possible to take records at any speed up to 5 m. per sec. Calibration photographs were taken at the beginning and end of each experiment. Stimuli were given by a coreless induction coil, in the primary circuit of which was a contact breaker operated by a cam on the driving shaft of the camera. Just maximal shocks were used throughout. The number of stimuli given to the tissue per second varied with the camera speed. In the

case of Figs. 4 and 5 stimulation was at the rate of six per second, in Figs. 6 and 7 at two per second. It was not found that the rate of stimulation affected the results. The contraction of the muscle was isometric. All the preparations were made from *Rana temporaria* in the winter, and the temperature was kept constant at about 15° C. during each experiment. It did not vary by more than two degrees from experiment to experiment.

The first preparation to be used was the ordinary sciatic-gastrocnemius. It was soon clear, however, that no accurate or reproducible results could be obtained with this muscle, owing to the complex character of the action potential, and to great variability in its point

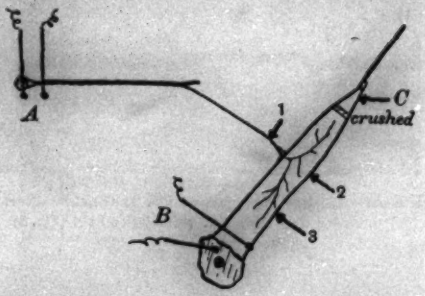

Fig. 2. Arrangement of electrodes on sartorius preparation.

of origin. This result was perhaps to be expected from the anatomical arrangement of the fibres. Generally a point just above the belly of the muscle was the first to become active, but often, as the muscle became fatigued, the point would shift, sometimes to such an extent that the sense of the action potential would be reversed, indicating that the first point to become active was nearer the grid electrode on the tendon. Nearly always a polyphasic action potential appeared in the course of fatigue. It was therefore decided to use the sartorius muscle and its nerve (Fig. 2). Electrodes 1, 2 and 3 and the stimulating electrodes *A* and *B* were made of silver wire 1·25 mm. in diameter coated electrolytically with silver chloride and fixed to ball and socket holders. The grid electrode was of the wick type. Usually the tendon end of the muscle was crushed in order to obtain monophasic action potentials and to increase their amplitude when the leading-off electrodes were close together. It was

not found that this treatment was detrimental to the tissue. The muscle was suspended in air and frequently irrigated.

When the preparation was first set up after equilibration in oxygenated Ringer's solution for 1 hour, a photograph (Fig. 3 *a*), was quickly

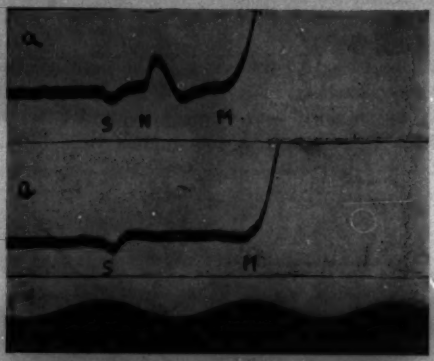

Fig. 3. Action potentials from fresh preparation. Time marker gives 180 d.v./s. *a*, leads 1 *G*; *b*, leads 2 *G*. Stimulation at *A* (Fig. 2).

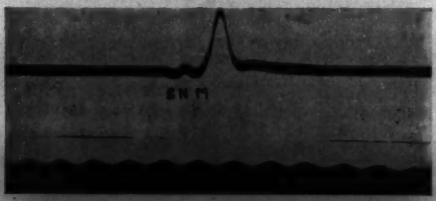

Fig. 4. Action potentials from fresh preparation, using lower amplification. Time marker gives 180 d.v./s. Leads 1 *G*. Stimulation at *A* (Fig. 2).

taken with leads 1 *G* (Fig. 2), closely followed (Fig. 3 *b*) by one with leads 2 *G*. Fig. 4 was taken at a lower speed and with lower amplification. The muscle-action potential, *M*, is simple in character and is preceded by the nerve-action potential, *N*, and the stimulus escape, *S*. It will be seen that in Fig. 3 *a* the distance *S*–*M* is slightly less than in Fig. 3 *b*. The probable reason for this is that in the sartorius preparation the

electrode 2 is on the side of the muscle opposite to the point of entry of the nerve: the fibres under it will receive the nervous stimulus slightly later than those connected by the nerve itself to electrode 1. In the gastrocnemius preparation, on the other hand, the point at which the nerve comes into contact with the muscle is not that at which the muscle is first excited. This explains Samojlov's results in which records obtained with either leads 2 *G* or 3 *G* (Fig. 1) gave a shorter duration *S–M* than those obtained with leads 1 *G*.

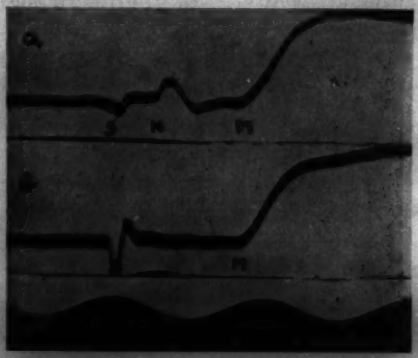

Fig. 5. Same preparation as Fig. 3 after stimulating for 3 min. at six stimuli per second. Amplification and time marker as in Fig. 3. *a*, leads 1 *G*; *b*, leads 2 *G*. Stimulation at *A* (Fig. 2).

It was then intended, as stated, to poison the muscle with curare; in the preliminary experiments, however, in which the apparatus was being adjusted and the preparation was unavoidably becoming fatigued, observation did not seem to verify Wieser's results. Attention was accordingly directed to this problem. The preparation was fatigued by indirect stimulation at the rate of six stimuli per second, the changes in the action potentials being meanwhile observed on the viewing screen. When the muscle action potential as seen on the screen had shrunk to one-tenth of its original amplitude, two photographs (Fig. 5) were taken as before. At this stage the contraction of the muscle was barely perceptible.

Results.

From a comparison of the second pair of photographs with those from the preparation when fresh, it is evident that there is no detectable increase in the distance *N–M* as measured from the beginning of the nerve-action potential to the beginning of the muscle-action potential. This result was obtained on all sartorius preparations.

Further experiments with the gastrocnemius showed that results similar to Wieser's could be obtained with this muscle when the electrodes were suitably placed. With electrodes 1 *G* or 2 *G* (Fig. 1), the delay

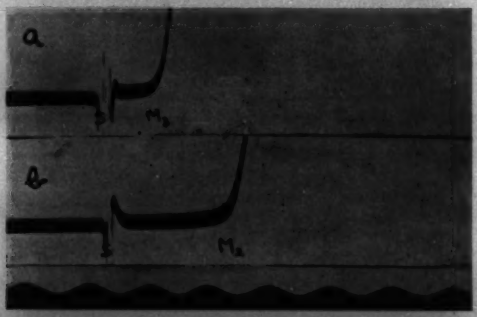

Fig. 6. Muscle action potentials from fresh preparation. Time marker gives 180 d.v./s. *a*, leads 3 *G*; *b*, leads 2 *G*. Stimulation at *B* (Fig. 2). Conduction rate 1·5 m. per sec.

in the fresh preparation was found to be greater than with the corresponding leads on the sartorius preparation. Further, this delay increased in fatigue by as much as 60 p.c. On the other hand, in some preparations, leading off from lead 3 quite close to lead *G* gave results similar to those obtained with the sartorius. It was suspected that these inconsistencies might be traced to changes in the rate of conduction in the muscle itself, for the nearer the proximal lead was to the point of first activity of the muscle, the less was the discrepancy between gastrocnemius and sartorius. The difficulty with gastrocnemius was, as has been mentioned, that the point of first activity often shifted in the course of an experiment.

A sartorius preparation was then stimulated directly through the leads *B* (Fig. 2), and a pair of photographs (Fig. 6 *a* and *b*) taken with

leads 3 *G* and 2 *G* respectively. In this way it was possible to avoid any error due to the spread of the stimulating current. The rate of conduction from lead 3 to lead 2 was measured by subtracting the distance *S–M* 3 from *S–M* 2 (Fig. 6). The muscle was slowly fatigued and photographs (Fig. 7) were taken when the action potentials were one-tenth their original size. In the case of the preparation from which Figs. 6 and 7 were taken, the rate of conduction when fresh was 1·5 m. per sec., and dropped in fatigue to 0·9 m. per sec. This agrees well with Wieser's experiments, in which the delay, which he thought due to the end-plate, was about 60 p.c. greater in the fatigued preparation than in the fresh one.

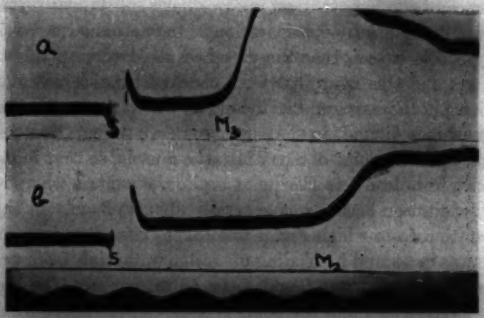

Fig. 7. Same preparation as Fig. 6 after stimulating for 5 min. at two stimuli per second. Amplification twice that in Fig. 6. Time marker gives 180 d.v./s. *a*, leads 3 *G*; *b*, leads 2 *G*. Stimulation at *B* (Fig. 2). Conduction rate 0·9 m. per sec.

The experiments on indirect stimulation were repeated with varying positions of lead 2 (Fig. 2). It was found that the delay increased as the lead was moved farther toward the pelvis away from the region of the muscle where nerve endings are found, and that the longer the delay was, the more it was affected by fatigue.

There can be little doubt that the delay measured by Wieser, Samojlov and others was the sum of the true delay and the muscle conduction time, though Wieser, at least, thought that he had made adequate control experiments. The changes observed in fatigue must similarly have been changes in the muscle itself.

Discussion.

The results of Forbes, Ray and Griffith in connection with the long delay experienced by a second impulse given to a motor nerve may be easily explained if it be supposed that in their experiments also the muscle conduction time was included in measurements of the delay. As the second impulse would stimulate the muscle in its relative refractory period, the muscular impulse would be conducted more slowly to the leading-off electrodes, thus causing an apparent delay in the appearance of the muscle-action current.

The constancy of the end-plate delay in fatigue weakens the case for the existence of the hypothetical intermediary substance; further doubt is cast upon its reality by consideration of the actual value of the delay. From Fig. 4 *a* it is seen that the distance *N–M* corresponds to a time of about 3σ, and this figure has been checked by observation of many preparations, both photographically and by measurement on the viewing screen. Now it is difficult in the sartorius-nerve preparation to bare the nerve right up to its point of entry into the muscle, so that in most cases the distance from lead 1 to the point inside the muscle where it may be presumed excitation occurs was generally about 1·5 cm. In the case of the preparation from which Fig. 4 was taken, the rate of conduction in the nerve was measured by leading off action potentials from two places, as for the muscle, and was found to be 15 m. per sec. The distance of 1·5 cm. between lead 1 and the point of action of the nerve would therefore be traversed in about $1{\cdot}0\sigma$; this time may be subtracted from the delay observed. The remainder, 2σ, may be divided into two parts: the first due to the latent period and conduction time of the muscle, and the second to some process yet to be accounted for. The amount due to conduction in the muscle must be small, since the increase in conduction time which has been shown to occur in fatigue would lead to the detection of a delay due to this source if it were greater than $0{\cdot}25\sigma$. Consideration of the latent period of the muscle raises the question as to what is the effective stimulus for the muscle. If, as Lapicque, for example, believes, the stimulus is merely the current due to the nerve-action potential change, it is unlikely that the moment at which the action current sets up a muscular impulse will occur sooner than the end of the rising phase of the action potential. In Fig. 4 the action potential of the nerve is diphasic, and the rising phase is curtailed for this reason: monophasic recording from the same nerve showed that the actual duration of the rising phase was approximately $1{\cdot}2\sigma$. This time may therefore be sub-

tracted from the delay, leaving 0·8σ unaccounted for. Apart from the possibility that there is a delay associated with a separate intermediate substance—a possibility not ruled out by these experiments—there are two alternative explanations of the small delay remaining. Either a slower conduction rate may be postulated for the terminal branches [Fulton, 1926], or it may be that the utilization time by the muscle of the nerve-action current is longer than 1·2σ. There is nothing to show which of these explanations is the true one, nor are they mutually incompatible. The question is discussed in some detail by Forbes, Ray and Griffith [1923, pp. 603–9].

The conclusion to be drawn from these results is that, if there is an intermediary substance, any function which it may be shown to have must be such as can be performed in a very short space of time. This renders unlikely the existence of a "receptive substance," and it would seem more satisfactory, therefore, to attempt to explain fatigue on the basis of changes which are known to occur in muscle, rather than on the assumption of changes which cannot be observed in a hypothetical substance.

The effect of curare, both upon indirect excitation as measured in these experiments, and upon the characteristics of the muscle response evoked by direct stimulation, should throw light on this problem.

Summary.

1. Measurements have been made of the delay in the transmission of activity from nerve to muscle by leading off the electrical changes in both to an amplifier and Matthews oscillograph.

2. Fatigue causes no increase in the delay when a sartorius-nerve preparation is used.

3. Observation of the changes in muscle conduction rate resulting from fatigue indicate that the changes hitherto attributed to an intermediary substance were changes in the characteristics of the muscle.

4. Values for the delay derived from experiments on the sciatic-gastrocnemius preparation are too high, being the sum of the true delay and the conduction time in the muscle.

5. In winter specimens of *Rana temporaria* at 15° C., the delay is never more than 2·0σ. Under these conditions the rate of conduction in the nerve is 15 m. per sec.

6. It is suggested that there is no need to postulate either a slower conduction rate in the terminal nerve fibres or the existence of a separate

substance in order to account for the delay, which is less than the duration of the nerve action potential.

It is a pleasure to record my deep gratitude to Mr Matthews for his advice and instruction and to Dr Rushton for his helpful criticism.

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THE REGULATION OF THE PYLORIC SPHINCTER.

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Considerable discussion has taken place as to the mechanism regulating the opening and closing of the pyloric sphincter since Cannon in 1907 enunciated his theory of the acid and alkali control. Unfortunately, as Alvarez [1928 *a*] points out, commentators have not infrequently quoted incorrectly and, further, have failed to note that Cannon had seen defects in his theory, as in certain conditions it did not explain all the facts.

An excellent summary of the literature on the control of the pylorus is given by Alvarez. In most of the investigations described, attention has been directed to the consistency or reaction of the foodstuff, and little or no regard has been paid to the analysis of the movements of the pyloric antrum and sphincter. Wheelon and Thomas [1920, 1922], however, have studied the relation between the sphincter and antral contractions in dogs by means of an enterograph introduced into the antrum. These observers show that the pyloric sphincter has cycles of rhythmic motility and that these cycles are coincident and of the same duration and rhythm as those of the antrum proper. "A phase of inhibition (maximal relaxation) manifests itself during the height of antral contraction. Following this, and while the antrum is finishing its contraction, the sphincter begins its positive phase and reaches the height of its contraction when the antrum is rapidly relaxing or has relaxed. Following this, the sphincter relaxes." They further suggest that the sphincter may act not only in relation to the antrum to aid in the propulsion of material from the stomach, but also as a barrier to the regression of chyme during the presence of a positive phase of the duodenum.

The co-ordination which exists between the movements of the antrum and the pyloric sphincter and the part played by the sphincter in regulating the passage of fluid may be studied by using the preparation described by McCrea and McSwiney [1926]. It has been possible to

separate the antrum from the body of the stomach without interference with the nerve or blood supply and to record simultaneously the contractions of the antrum and the amount of fluid flowing through the sphincter. The method has distinct advantages, as there is no disturbance of the pyloric sphincter.

METHOD.

Dogs have been used in these experiments. The animal was first anæsthetized with ether, sustained anæsthesia being obtained by intravenous chloralose (0·075 g. per kg.). A second injection was occasionally necessary some 2–4 hours later. The abdomen was opened through a mid-line incision, and the body of the stomach separated from the pyloric antrum. The distal end of the body was then closed by a running Czerny-Lembert suture. A wide-bore cannula was inserted and tied into the antrum. Alternatively, a ligature may be passed through the greater and lesser omentum close to the curvatures of the stomach at the incisura, and tied tightly round the junction of the antrum and the body; a small opening is made into the antrum near to the point of ligature, and a cannula inserted and securely tied.

A method suggested by Babkin [1931] was used in several of the earlier experiments. In the region of the incisura a longitudinal incision 1 in. long was made through the seromuscular coat to the mucosa midway between the greater and lesser curvatures, and a tube of mucous membrane was separated from the seromuscular coat. A cannula was inserted into the tube which was then tied off.

The duodenum was divided transversely 1½ in. from the pylorus. A wide cannula was tied into the proximal end, 1 in. distal to the pylorus. Rubber tubing connected the cannula with a U-tube manometer. The fluid, escaping from the antrum, flowed into one limb of the manometer, and was recorded on the kymograph by the movement of the waterfloat; the level of the fluid in the manometer may be lowered after a series of contractions to its resting level.

An interval of approximately 30–60 min. was allowed to elapse after the operation; the cannula in the antrum was then attached to the tube leading from a reservoir supported 2 ft. above the level of the antrum. A valve was placed at the lower end of the tube, and this allowed fluid to flow into the antrum only during the relaxation phase. A tube, attached to a tambour for recording antral contractions, was connected to the inflow tube below the valve.

The tap of the reservoir was opened, and the rate of inflow was varied

by inserting capillary tubes of different calibres into the rubber cork of the reservoir. The reservoir is placed at a height above the animal to ensure "diastolic" filling. The pressure was never sufficient to open the sphincter, which is able to resist comparatively high pressures. The pressure of the fluid does not therefore determine the rate of escape of fluid from the antrum, but merely ensures adequate filling.

Experimental results.

Simultaneous records of the contraction of the antrum and the amount of fluid flowing through the sphincter were first obtained using 0·9 p.c. sodium chloride solution as the inflow fluid. Slight movements are observed even before fluid fills the antrum, but well-marked contractions do not occur until the antrum is distended [Ducchesi, 1913]. The amplitude of contractions varied in different animals, but the height of the contraction wave was remarkably constant in the same animal for any given rate of inflow. The height of contraction was found to vary with the inflow, and, if the rate of inflow was increased, the amplitude of contraction increased. The form of the antral contraction was observed to be constant, and consisted of an even ascent and descent of the curve, except for a concavity on the rising limb which marks the opening of the pyloric sphincter. Small superimposed respiratory waves are usually present. A large contraction occurring in a series of regular waves was generally preceded by a longer period of diastole. The frequency of contraction remained remarkably constant throughout the experiment, and little or no change was observed on altering the inflow.

The saline solution was expelled from the antrum through the sphincter in spurts or jets which synchronized with the antral contractions. The outflow of fluid from the antrum was recorded, and, as fluid only escaped during the antral systole, a step-like tracing was obtained. If fluid enters the antrum too rapidly, or if, owing to the bad condition of the animal, the sphincter is not able to hold back the fluid, the tracing illustrating the outflow becomes a continuous rising line. The state of the pyloric sphincter is therefore indicated by the type of outflow curve.

It has previously been observed that the height of the antral contraction varies with diastolic filling. In the same way, the amount of saline solution expelled with each contraction varies with the inflow. With a constant rate of inflow, the amounts expelled at each contraction vary slightly from one another, but increase in inflow causes a greater antral contraction with a corresponding increase of outflow.

TABLE I.

Inflow	Height of contractions (antrum) cm.	Frequency of contractions (antrum) per 3 min.	Average outflow per contraction c.c.
Small	2·3–3·2	12	4·8
Medium	2·4–4	14	7·7
Large	2·5–5·5	16	8·9

Fig. 1. Tracing to show relation between (*P*) contraction of pyloric antrum and (*O*) outflow of saline through the pyloric sphincter. Time interval 1 sec.

The size of antral contraction is not, however, the only factor regulating the outflow, as not infrequently a larger amount of fluid is expelled with a small contraction than with a large contraction. It would appear, therefore, that the amount of outflow with a constant inflow depends on (*a*) the contraction of the pyloric antrum, and (*b*) the relaxation of the pyloric sphincter. It is clear that the extent and duration of the relaxation period of the sphincter must be the principal factor determining the outflow.

Tracings illustrating the relation between the movements of the antrum and the pyloric sphincter (Fig. 1) have been obtained. The time

intervals will, of course, vary in each experiment according to the frequency of contraction and other factors.

In Table II a series of measurements obtained with saline is recorded. It will be observed that the outflow commenced some 2–4 sec. after the onset of the contraction of the pyloric antrum. The outflow usually lasted for some 4–6 sec. and ceased before the antrum relaxed. It may, therefore, be stated that the pyloric sphincter relaxes for a period of approximately 4–6 sec. during the period of antral contraction. It must be emphasized that in all our experiments the sphincter remained contracted except for short periods of the antral cycle. The measurements given in Table II are not strictly accurate, as a lag occurs between the contraction of the antrum and the reception of fluid in the manometer.

TABLE II. Saline, constant inflow.

	Height of contraction (antrum) cm.	Amount of outflow c.c.	Time of outflow sec.	Contraction (antrum) Ascending sec.	Contraction (antrum) Descending sec.	Interval between contraction and outflow sec.
Dog 1	6·3	4	5	8·5	7	2·5
	6·3	4·4	6	10	7·5	3·5
Dog 2	3·7	1·2	5	9	8	3·5
	4·6	2·4	8	12	6	3
Dog 3	3·2	3	3·5	4	4·5	3
	2·1	3	2	4·25	6	4
Dog 4	4·5	2·8	4	8·75	5	3·75
	3·7	2·8	4	7·75	4	3

A series of experiments has been made on the effects of various fluids upon the activity of the sphincter pylori. It is usually stated that fats leave the stomach at a slower rate than proteins, and again that solid and semi-solid foods empty less quickly than liquid. Considerable variation in the rate of outflow of liquids from the stomach is further described. Acid fluids empty at a slower rate than neutral solutions, and alkaline solutions empty at a rate which is between that of acids and neutral solutions [Alvarez, 1928 *b*].

To determine whether acid, alkali, salt, organic acid or viscous solutions influence the state of the pyloric sphincter, the passage of the following fluids through the pyloric antrum was investigated.

I. Sodium chloride, 0·9 p.c.; sodium chloride, 25 p.c.; distilled water; glucose, 25 p.c.; hydrochloric acid, 0·18 p.c. in normal saline; sodium bicarbonate, 0·25 p.c. in normal saline.

II. Olive oil; gum acacia, 10 p.c. and 25 p.c. in normal saline.

III. Organic acids, 2 p.c. solutions; lactic, malic, succinic, tartaric and caproic acids.

Fig. 2. Tracing to show relation between (*P*) contraction of pyloric antrum and (*O*) outflow of fluid through the pyloric sphincter. *A*, saline; *B*, alkali; *C*, acid. Time interval 30 sec.

The contractions of the pyloric antrum and the outflow of fluid through the sphincter were first recorded, using 0·9 p.c. sodium chloride solution. At the end of this initial experiment the inflow fluid was

changed over, and comparable tracings were taken with one of the test solutions. Each preparation was used to test three or four solutions.

TABLE III.

Fluid	Height of contraction (antrum) cm.	Amount of outflow c.c.	Time of outflow sec.	Contraction (antrum) Ascending sec.	Contraction (antrum) Descending sec.	Interval between contraction and outflow sec.
Saline	6·3	4	5	8·5	7	2·5
Saline	6·3	4·4	6	10	7·5	3·5
Alkali	4·4	5	6	10	5	2
Alkali	5·7	5	6	13	7	4
Acid	5·5	4·8	6	12·5	6·5	4·5
Acid	4·1	6·4	7·5	12	5	4·5

Two readings are given for each fluid, the first set of readings being taken shortly after the start, and the second towards the end of the experiment. The inflow was constant throughout.

The results obtained with the fluids of Groups I, II, III were in no way different from those obtained with saline. Records and measurements made using saline, alkali and acid are given in Fig. 2 and Tables III and IV. The base line of the antral contractions remained remarkably constant, demonstrating that there was no appreciable change of tonus. When the inflow fluid is first changed over, the antral contractions and the outflow curve occasionally become irregular for a short interval. The preparation, however, soon settles down, and regular records are obtained.

TABLE IV.

Fluid	Average height of contraction (antrum) cm.	Frequency of contraction (antrum) per 3 min.	Average outflow c.c.
Saline	5·9	4	4·3
Saline	6·7	4	3·8
Alkali	7	5	3·1
Alkali	6·8	5	4·5
Acid	6·5	6	4·8
Acid	7·2	6	4·3

Two readings are also given in this table for comparison as in Table III. The inflow was constant.

The regular step-like character of the outflow showed that the sphincter remained closed between the systoles of the antrum. If the rates of inflow were increased, a greater amount of fluid, as has been previously pointed out, was ejected at each contraction. With high rates of inflow, the step-like character of the outflow curve was lost, indicating that the outflow continued, though usually at a diminished rate during

the diastole of the antrum. With viscid solutions, the outflow curve, as might be expected, was not as clean cut as for other solutions.

It must be emphasized that in no instance was the passage of fluid from the antrum to the intestine influenced by the type of solution. In some experiments, however, an increase in the frequency of antral contractions was recorded with the different inflow fluids.

Discussion.

By the use of the isolated pyloric antrum preparation with intact nerve and blood supply, as described in this paper, it is possible to demonstrate that the movements of the pyloric antrum and pyloric sphincter are related. Criticism may be advanced that by the introduction of a valve between the reservoir and pyloric antrum, the condition of the antrum becomes abnormal. In preliminary experiments the valve was omitted, and results similar in all details were obtained, but the amount of fluid ejected from the antrum with each contraction varied considerably. It will be realized that in the normal stomach as digestion proceeds, the stomach becomes more tubular and the prepyloric sphincter is definitely formed [Cathcart, 1911]. Indeed, with a barium meal it is possible to see the antrum full of barium apparently completely separated from the remainder of the stomach. The valve was therefore introduced to imitate the effects produced by the prepyloric sphincter.

We are able to confirm the previous observations of Wheelon and Thomas [1920, 1922] that the movements of the antrum and sphincter are related, but we disagree with the statement that while the antrum is finishing its contraction, the sphincter begins its positive phase and reaches the height of its contraction when the antrum is rapidly relaxing or is relaxed.

Our results show that the pyloric sphincter is normally contracted, as can be seen from the step-like form of the outflow record. Following the contraction of the antrum, the sphincter relaxes and returns to its state of contraction in some 5–6 sec. The relationship between the contraction and relaxation of the sphincter depends mainly on the rate of antral contraction. According, however, to Wheelon and Thomas, the sphincter is usually relaxed and only contracts for a period following the antral contraction to act as a barrier to the regression of chyme during the presence of a positive phase of the duodenum. It is extremely difficult to see how the stomach could hold fluid if the normal state of the sphincter were relaxation. The rhythmic contractions of the pyloric sphincter recorded by Wheelon and Thomas are, in our opinion, due

at least in part to the stretching of the muscle fibres by the insertion of a balloon. For in the animal, as in isolated preparations, stretching of the muscle fibres acts as a stimulus for rhythmic contractions.

It has also been possible to demonstrate that the different solutions employed have little or no influence upon the pyloric sphincter when allowed to flow into the antrum. It has been pointed out that acid solutions are stated to empty at a slower rate than neutral and alkaline solutions at a rate between acid and neutral solutions. Again, Carnot and Chassevant [1905] have shown that the rate of emptying of hyper- and hypotonic solutions of glucose increased as the solutions approached isotonicity. Magee and Reid [1931] and McSwiney and Spurrell

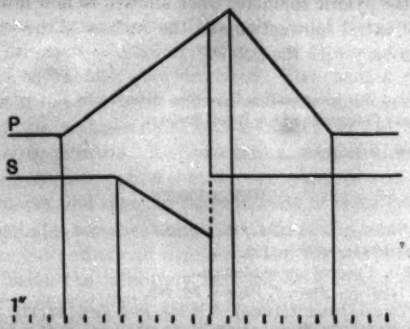

Fig. 3. Diagram to illustrate the relation between (*P*) contraction of the pyloric antrum, and (*S*) the relaxation of the pyloric sphincter.

[1932] have also suggested that osmotic pressure of the stomach contents may prove to be an important factor in regulating the emptying of the stomach. As the pyloric antrum and the sphincter are, according to our experiments, insensitive to factors which other authors state either increase or decrease the emptying time of the whole stomach, it follows that the body of the stomach must react by increase or decrease of tone according to the fluid or foodstuff it holds.

It is interesting to note the similarity which exists in some respects between the pyloric antrum and the right or left ventricle, as is evidenced by the rhythmicity of the antral contraction, the variation in height of contraction with diastolic filling, and the increase in outflow with corresponding increase in inflow. On the other hand, variation in inflow causes little or no change in the frequency of contraction.

Summary.

Experiments are described in this paper which demonstrate the relationship between the movements of the pyloric antrum and sphincter in regulating the passage of fluid from the stomach to the duodenum. The fluid was expelled from the antrum through the sphincter in spurts which synchronized with the antral contraction.

The sphincter, as judged by the outflow from the pyloric antrum, is normally contracted, and relaxes some 2–4 sec. after onset of the antral contraction for a period of some 6 sec. Acids, alkalis, hypo- and hypertonic solutions and solutions of different organic acids have little or no influence on the pyloric sphincter when allowed to flow into the antrum. The height of antral contraction and the outflow of the antral contents were found to vary with the inflow.

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FACTORS INFLUENCING THE ACTIONS OF CORPUS LUTEUM EXTRACTS ON THE RABBIT'S UTERUS.

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In a previous communication [Robson and Illingworth, 1931] the effects of injection of various extracts of bovine corpora lutea into ovariectomized rabbits were described. The changes resulting in the uterus were studied in detail with special reference to the specific proliferation of the endometrium and inhibition of the reaction of the musculature to pituitrin. Evidence was presented which could best be explained on the assumption that the two reactions were due to different luteal hormones and that partial separation of these had been effected. It seemed at the time that crude extracts of the corpus luteum would always give both effects on the uterus, and Knaus [1930] had actually suggested using the inhibitory reaction as a means of standardizing extracts. Tausk *et al.* [1931] subsequently also showed that corpus luteum extracts did not cause both proliferation and inhibition with any degree of regularity, and that multiple doses of an extract might produce less inhibition than the original dose. They came to the conclusion that the correlation between the proliferation and inhibitory phenomenon was too small to justify the assumption that they were caused by the same factor. Clauberg [1931] suggested that the α hormone (œstrin) could prevent the development of the inhibitory reaction, and this point of view is further developed by his colleagues Hartmann and Störring [1931], although no exact experimental data are given.

During the last eighteen months we have obtained additional data. The effect of dosage on the development of the uterine reactions has been studied so as to allow of a more exact evaluation of the "separation" experiments. Methods have been evolved for the further purification of the corpus luteum extracts. Additional experiments also on the relationship of α (œstrin) to the changes brought about in the uterus by the luteal extracts have been performed.

Technique.

Seventy-five mature female non-pregnant rabbits, each weighing about 2 kg., were used in these experiments. The animals were of various breeds, as we found it difficult to procure animals of the same breed in sufficient numbers; but we have satisfied ourselves that the results obtained were not due to variations occurring between different breeds of rabbits. The animals were oöphorectomized and the absence of pregnancy or pseudo-pregnancy was established at the operation. Injections were administered over a number of days. The animals were then killed by a blow on the occiput or by injection of air into an ear vein. One horn of the uterus was placed in Bouin's solution and sections prepared from it; the other was used for experiments *in vitro*. Ringer-Locke solution was used in all cases in 100 c.c. containers. Temperature (37·5° C.) and oxygen supply were maintained constant. Records on smoked drums were taken with lightly balanced lever. The dose of pituitrin used in the majority of experiments was 5 units. The contractility of the uterus was in all cases tested by the subsequent addition to the bath of 0·1 c.c. of 1/1000 adrenaline solution.

The standard extracts were prepared as follows: cow's ovaries were dissected within a few hours of the animals being slaughtered. Only solid corpora were used, and these were finely minced and extracted in the cold with two volumes of 95 p.c. ethyl alcohol for at least 24 hours in each extraction. The alcoholic extract was evaporated to dryness *in vacuo* and the residue extracted thoroughly with 100 c.c. portions of ether. The tissue, after alcohol extraction, was dried and extracted in a Soxhlet with ether, and the two ether extracts combined and evaporated to dryness. The viscous oil was then extracted with ethyl acetate. This on evaporation yielded a dark brown oil which solidified on standing. The yield was about 25 g. per kg. of corpora lutea.

Results.

The results obtained with the standard extracts are grouped according to dosage in Table I. The degree of proliferation is shown in column 3; +++ represents a degree of proliferation equivalent to that observed on the 8th day of pseudo-pregnancy in an intact rabbit, while + indicates the minimal degree of proliferation clearly demonstrable. Column 4 shows the reaction of the musculature to pituitrin; + indicates that inhibition had developed, in other words, that pituitrin (5 units in 100 c.c. Ringer-Locke solution) was without effect or caused relaxation.

TABLE I.

Extract No. β	Animal No. Ra	Proliferation	Inhibition
Dose 1–2 c.c.			
15	59	+++	+
20	118	+++	–
23	158	+++	–
24	163	+++	+
35	245	++	–
36	253	++	+
41	284	+++	–
42	286	++	–
48	294	+++	–
54	310	++	+
Dose 2–3 c.c.			
22	122	++	+
29	224	++	–
30	206	+++	+
35	242	+++	–
50	299	+++	+
51	308	+++	+
52	303	+++	–
53	302	++	+
58	321	+++	+
Dose > 3 c.c.			
29	230	+++	–
41	265	++	+
44	276	+++	+
44	298	+++	+
24	163	++	+
48	300	+++	+
35	246	+++	+
52	304	+++	–

It will be seen that in all cases the extract caused some degree of proliferation. Inhibition, however, was not obtained in all the experiments, but it will be noticed that the incidence of inhibition increases with the dose administered. This is more clearly shown in Table II which correlates the dosage with the percentage of experiments in which both effects were obtained. It will be seen that, while a dose of 1–2 c.c. caused inhibition in only 40 p.c. of experiments, the larger dosage (*i.e.* doses greater than 3 c.c.) were successful in causing inhibition in 75 p.c. of experiments.

TABLE II.

Dosage c.c.	No. of animals	Proliferation and inhibition p.c.	Proliferation only p.c.
1–2	10	40	60
2–3	9	66·6	33·4
3	8	75	25

As it had been found previously that purification of the extracts by partitioning between volatile media (to which we shall make further

reference in the discussion) was attended by some degree of dissociation of the two effects, further methods of purification were evolved in the hope that a more definite separation would result.

(i) *Partition between 66 p.c. acetic acid and petroleum ether* (B.P. 40–60° C.). In a preliminary experiment 2 c.c. of extract β18 were dissolved in 10 c.c. petroleum ether and the solution extracted thoroughly with like portions of 66 p.c. acetic acid. The solvents separated fairly readily and the extraction was continued until the acetic acid remained colourless after shaking with the petroleum ether solution.

On evaporating the acetic acid fraction to dryness under reduced pressure, a greenish brown gum remained which, when injected into rabbit 93, caused good proliferation (+++) and complete inhibition. The petroleum ether fraction had retained none of the active substance.

While no separation of the active factors was effected, the volume was reduced very considerably and a colloidal solution in water produced. Therefore, the method was considered to be of value in so far as purification of the crude extracts was concerned and has been used in a number of experiments. In no case has the preparation produced been desiccated, so that dry weights for the unit cannot be given, but 1 g. of the "gum" contains about 20 rabbit units.

TABLE III. To show the effect of the acetic acid fraction on the uterine endometrium and muscle.

β	Animal No. Ra	Proliferation	Inhibition
18	93	+++	+
18	96	+++	–
20	107	++	–
36	254	++	–
48	300	+++	+
54	310	+++	+
55	309	+	+

(ii) *Partition between methyl alcohol and petroleum ether* (B.P. 40–60° C.). 2 c.c. of extract β23 were dissolved in petroleum ether and the solution extracted twelve times with methyl alcohol, until, that is, the alcoholic phase remained colourless. The combined alcohol fractions were washed once with a small volume of petroleum ether and evaporated to dryness *in vacuo*. The residue was dissolved in a convenient quantity of maize oil and injected into Ra 149. Section of the uterus showed good proliferation, while a marked contraction followed the addition of pituitrin to the uterus *in vitro*. This experiment was repeated with β24 and β25. The results are shown in Table IV.

TABLE IV. To show the effect of the methyl alcohol fraction on the uterine endometrium and muscle.

β	Animal No. Ra	Proliferation	Inhibition
23	149	+++	–
23	159	+++	–
24	164	++	–
25	182	++	–

(iii) *Partition between* 45 *p.c. ethyl alcohol and petroleum ether* (B.P. 40–60° C.). Separation of the solvents was extremely slow and tedious, and the addition of a drop of HCl was without effect. The alcohol fraction on evaporation yielded a minute amount of inactive material, while the petroleum ether fraction had completely retained its activity as shown by injection into Ra 60.

(iv) *Partition between* 50 *p.c. ethyl alcohol and chloroform.* In this experiment, which was carried out as described above, the chloroform fraction on injection into Ra 74 produced good proliferation and complete inhibition, while the portion soluble in alcohol was without activity.

(v) *Partition between* 50 *p.c. ethyl alcohol and benzene.* Separation of the solvents was exceedingly slow owing to the formation of an emulsion. The product obtained from the alcohol fraction (used in aqueous suspension) was almost inactive, whereas the benzene fraction appeared to have lost very little of its activity.

(vi) *Partition between* 66 *p.c. acetone and petroleum ether.* 2 c.c. of extract β16 were dissolved in petroleum ether and the solution extracted with an equal volume of 50 p.c. acetone. An emulsion was formed and therefore the concentration of the acetone was increased to 66 p.c. when separation of the solvents took place readily. On injection of the acetone-soluble fraction into Ra 79 it was found to be without activity, while the petroleum ether fraction caused good proliferation and inhibition when injected into Ra 80.

Modification of methods of initial extraction. During the summer months of 1931 it was found difficult to obtain supplies of corpora lutea from Glasgow in sufficient amount and consequently the glands were obtained from Holland preserved in acetone. On arrival in the laboratory the acetone was drained off the corpora, the tissue minced and extracted with 95 p.c. alcohol twice for 24 hours. The solvents were combined, evaporated *in vacuo* and the preparation carried out as hitherto described.

All the extracts prepared from these supplies were found to be completely inactive, and on enquiry being made in Holland it was found that the corpora lutea had been stored in acetone for some considerable time.

In view of this, therefore, the effect of acetone extraction was investigated more carefully as follows: Fresh corpora lutea were finely minced and extracted in the cold for several days and in some cases for several weeks. The acetone was filtered off and evaporated *in vacuo*. The residue was extracted with ether and the ether soluble material used for injection. The results obtained with a number of such extracts are shown in Table V.

TABLE V. To show the effect of an acetone extract of bovine corpora lutea on the uterine endometrium and muscle.

Period of extraction days	β	Weight of corpora lutea g.	Animal No.	Proliferation	Inhibition
4	31	500	Rb 3	+++	–
4	33	300	Rb 5	+++	–
6	37	250	Ra 257	++	–
2	39	240	Ra 260	++	+
2	43	275	Ra 273	+	+
40	46	150	Ra 305	+++	–

It will be seen that although large quantities of tissue were extracted (the minimal amount being equivalent to 4 c.c. standard extract) and that good proliferation resulted in five out of six experiments, yet inhibition was only obtained in two out of six.

Extraction with methyl alcohol was investigated, but little evidence was obtained to show that it had any advantage over ethyl alcohol as an extracting medium. It seemed rather, that prolonged treatment with this solvent inactivated the inhibitory factor. Extraction of corpora lutea for 2–3 days produced an extract which caused both proliferation and inhibition when injected in the usual way, while the extract obtained after treatment of the tissue for periods of 10 days or more caused only proliferation.

In view of the statement of Fevold, Hisaw and Leonard [1932] to the effect that by means of acid alcohol extraction and subsequent fractionation, it is possible to produce a number of active principles of the corpus luteum, it was decided to apply their method in an attempt to produce extracts which would bring about one or the other of the effects described herein.

Fresh bovine corpora lutea were finely minced and refluxed with two volumes of acid alcohol (98 c.c. ethyl alcohol + 2 c.c. HCl) twice, for 1 hour in each extraction. The tissue was completely disintegrated by the boiling acid alcohol which was removed by filtration through muslin; the dark brown solution was neutralized with sodium bicarbonate and the precipitate which formed was redissolved and the neutralization repeated.

The solution was evaporated to dryness *in vacuo* at a temperature below 45° C. and the residue extracted with alcohol; this was then removed under reduced pressure and the residue extracted with ether. Phosphatides were precipitated with acetone and the fraction soluble in acetone injected into Ra 313 and Ra 315. In each case the uterus showed slight proliferation (+) on section and no inhibition. A preparation from the same batch of corpora lutea carried out in the normal manner produced an extract which caused good proliferation with complete inhibition. This was repeated with a fresh batch of corpora lutea, and the extract obtained by the method of Hisaw was found to be almost inactive (Ra 329; proliferation +, inhibition negative). In every case the amount of extract administered to each animal was slightly greater than the usual dose of standard extract.

Relationship of α (œstrin) to the action of the luteal extract. It has been previously shown that the administration of α hormone (3000 m.u.) concomitantly with corpus luteum extract in oöphorectomized animals does not interfere with the development of the typical endometrial picture (progestational proliferation), nor with the inhibitory reaction.

It still was possible that the administration of α over a more prolonged period before the injections of corpus luteum extract would influence the subsequent effect of the extract on the uterine musculature as has been actually suggested by Hartmann and Störring [1931]. A series of experiments was carried out to investigate this possibility.

A number of animals were injected with 10 m.u. of an oily preparation of α per day, the first injection being given on the day following oöphorectomy. The α hormone was administered for 6 or 7 days and this was followed by the injection of corpus luteum extract, in doses similar to those used in previous experiments, over the usual period of 3 days. The animals were then killed and the state of the uterine endometrium and muscle determined. The results are figured in Table VI.

TABLE VI. To show the effect of α (œstrin) on the reaction of the uterine muscle and endometrium to the corpus luteum extract.

β	Dose c.c.	Proliferation	Inhibition	Animal No. Ra
35	2·7	+++	+	243
36	2·4	+++	+	262
36	2·4	+++	+	275
42–44	1·8	+++	−	287
42–44	3·6	+++	−	290
42–44	3·6	+++	−	291
48	3·6	+++	+	319
50	3·6	+++	+	322
52	3·6	+++	−	323

It will be seen that in each of the nine animals so treated a positive endometrial reaction was obtained. In addition, five out of the nine (or 55 p.c.) showed inhibition of the reaction to pituitrin. This result is not significantly different from that obtained in experiments where no α was given and where the injection of corpus luteum extract commenced immediately after oöphorectomy. It must therefore be concluded that under the given experimental conditions, the α hormone does not adversely affect the development of the inhibitory reaction.

Discussion.

The experiments described in this paper show in the first instance, that when an extract of the corpus luteum is injected into oöphorectomized rabbits some degree of correlation exists between the effects of such an extract on the uterine endometrium and muscle respectively; the degree of correlation is dependent on the dosage and increases in proportion to the amount administered. Nevertheless even with large doses there is still a significant number of experiments in which the uterus showed a positive reaction to pituitrin.

Tausk *et al.* [1931] have recently obtained similar results working on immature animals injected for 7–8 days with α (œstrin) previous to the administration of the luteal extracts and record the actual sensitivity of the uterine muscle to pituitrin in quantitative terms. No data are given for the sensitivity of animals treated with œstrin alone, so that apparently they have no standards of comparison. Our experience (with oöphorectomized and non-oöphorectomized mature animals) suggests that there exist great variations in the reactivity of the uterine muscle to pituitrin in the absence of any luteal activity, and for this reason we have attached significance only to those results in which the uterus failed to react to large doses of pituitrin. We are not satisfied that the failure of the uterus to react to small doses of pituitrin is necessarily due to an action of the luteal hormones.

From the given data it seems possible that an additional factor is involved in the production of the inhibitory reaction. Clauberg [1931] has indeed suggested that the amount of α administered is the decisive factor in determining whether inhibition develops, and both the α present in the extracts and the amount administered in the preliminary period in immature animals have to be considered; this point of view is further developed by his collaborators Hartmann and Störring, although no exact experimental data are given. This hypothesis is not supported by

our experimental material, for it has been shown that the administration of doses of α similar to those used by Clauberg *et al.*, and over a similar period, produces no appreciable effect on the development of inhibition. It must further be emphasized that our own extracts contain little α hormone (< 1 m.u. per rabbit unit corpus luteum extract), so that variations due to the α content of the preparations have, to all practical purposes, been eliminated. This conclusion is supported by experiments published elsewhere [Robson, 1932], where it is shown that the injection of 10 m.u. of α per day not only does not interfere with the development of the inhibitory phenomenon, but is actually necessary for the action of the luteal hormone on the uterine musculature in non-oöphorectomized animals injected with large doses of anterior lobe tissue.

The evidence we have previously advanced in which the corpus luteum and α hormones were administered simultaneously in oöphorectomized animals, also shows that α does not interfere with the inhibitory reaction.

Although the two reactions of the uterus (proliferation and inhibition) occur together in the majority of experiments, there is nevertheless evidence that they may be due to separate factors. In a previous paper [Robson and Illingworth, 1931] the results of experiments in which extracts of the corpus luteum were partitioned between 50 p.c. alcohol and petroleum ether were given, and the data now presented make it possible to estimate more accurately their significance. The results obtained are shown in Table VII.

TABLE VII.

		50 p.c. alcohol			Petroleum ether		
β	Dose c.c.	Animal No.	Proliferation	Inhibition	Animal No.	Proliferation	Inhibition
12	3	36	+	++	39	++	–
12	3	Not used			46	+++	–
12	3	42	++	–	41	+++	+
16	2	65	++	+	63	+++	–
16	2	67	+++	+	66	+++	+
16	2	70	++	+	69	+++	–
18	2·5	90	++	–	Not used		
14	3	49	++	+	48	+	–
14	3	50	+++	+	54	+++	–
14	2	56	+++	–	82	+++	+
17	1·5	81	+	–	84	++	–
17	1·5	85	++	+			

It will be seen that with the fraction soluble in 50 p.c. alcohol inhibition was obtained in seven out of eleven experiments, *i.e.* 63 p.c. of cases: with the petroleum ether fraction inhibition was only obtained in three out of ten experiments, *i.e.* 30 p.c. of cases. According to expectation the dose used should have given inhibition in 50 p.c. of experiments, the

results therefore suggest that the 50 p.c. alcohol phase contains about twice as much of the inhibitory factor as the petroleum ether fraction, yet the degree of proliferation with the petroleum ether fraction is more marked than that given with the alcoholic fractions. These results suggest to us that the two actions on the uterus can be referred to separate factors of the corpus luteum, but definite proof will only be obtained when a complete separation is accomplished.

While no advance has been made in the actual separation of the active factors of the corpus luteum (should two exist) a method has been evolved whereby a considerable purification of the standard extract can be quickly and conveniently effected.

By distributing the extract between 66 p.c. acetic acid and 40–60° C. petroleum ether, the weight of the extract is reduced from about 2 g. to 50 mg., and by more thorough extraction may be further reduced to 30 mg. without loss of activity. Allen's method [1930], although effective in obtaining a similar degree of purity, is long and tedious and considerable losses are sustained.

The effect of methyl alcohol extraction and separation is not clearly understood. In less than 20 p.c. of the total number of cases in which methyl alcohol was used, were both proliferation and inhibition obtained. A similar effect was obtained with acetone. The weights of tissue originally extracted with these solvents were, as a rule, considerably greater than those which yielded 1 rabbit unit by the standard method. The effect on the endometrium showed that active extracts were being produced. These experiments therefore appear to support the point of view that two factors are present in the corpus luteum, although, again, no complete separation was effected in these experiments.

The method of extraction described by Fevold, Hisaw and Leonard [1932] was investigated and found to produce an extract devoid of almost all activity. In view of the fact that these authors claim to obtain 30 rabbit units of the proliferative hormone per kg. of sow corpora lutea, and we adhered to their method exactly, our results can only be explained by the fact that bovine corpora lutea were used by us.

Standardization. Whatever decision will ultimately be arrived at as regards the existence of two luteal factors, it seems to us fairly evident that the inhibitory reaction is not a satisfactory test for the progestational hormone of the corpus luteum.

The progestational reaction of the endometrium gives more reliable and constant information as to the hormone content of a given extract. We have arrived at this conclusion from experiments performed on

mature oöphorectomized animals using a modification of the method originally devised by Corner and Allen [1929]. We have had no experience of the test as performed on immature animals, but from the published data cannot see that it has any advantages over that performed on the mature rabbit.

SUMMARY.

The action of standard extracts of the corpus luteum on the uterus of mature oöphorectomized rabbits was determined, with special reference to the effects on the endometrium (progestational proliferation) and the muscle (inhibition of the pituitrin reaction).

With a dose of 1 c.c. or more of the standard extract, progestational proliferation was obtained in nearly all cases; the frequency with which inhibition occurred varied with the dose injected, a larger dose being more often followed by the inhibitory reaction; even with comparatively large doses, however, 25 p.c. of the experiments showed the endometrial reaction without the effect on the muscle.

A method is described which allows of the further purification of the standard extract; the material so obtained produces the full effect on the endometrium in doses of less than 50 mg. and gives a colloidal solution when shaken up with water.

The effect of the α hormone (œstrin) on the reactions of the uterus to the corpus luteum extract has been further investigated. The α hormone does not appear to interfere with the development of the inhibitory reaction.

The evidence suggesting that the inhibitory reaction is due to a separate luteal hormone is discussed.

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THE ACTION OF PITUITARY POSTERIOR LOBE EXTRACTS ON DIFFERENT PARTS OF THE CIRCULATORY SYSTEM.

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The action of pituitary posterior lobe extracts on the circulation has received considerable attention, but has not been fully explained. All investigators are agreed that an intravenous injection of pituitrin produces constriction of the splanchnic vessels with a rise of systemic blood-pressure, but opinions differ as to how the other parts of the vascular system and the heart are influenced. Difficulties may exist in the fact that different species of animals do not behave in the same manner, so that it is not possible to transfer the results obtained from one to the other; on the other hand, they may arise from the fact that different investigations have been carried out under experimental conditions not altogether comparable with one another. Results obtained from observations on isolated organs, from perfusion of isolated parts of the vascular system or from the heart-lung preparation, cannot without reserve be applied to the intact animal. When therefore, for example, Dale [1909] or Gunn [1926] finds with the isolated heart that pituitrin constricts the coronary vessels, or when Anrep and Stacey [1927–8], Roessler [1930] and others describe its action on the heart-lung preparation, these observations cannot without further evidence be transferred directly to the conditions which obtain in the intact animal. It is further necessary, in the case of any effect on isolated tissues or organs, to be certain that it is due to an essential hormone in the extract and not to accidental accessory constituents, such as histamine. Further, now that it is known that the extract of the pituitary posterior lobe contains more than one active principle, it is important to know, especially with respect to certain effects, which of the known principles is responsible.

The present paper is an attempt to explain the effect of pituitary preparations upon the circulation by describing in the first place investigations on anæsthetized but otherwise intact animals, and in the second place experiments on isolated tissues or perfused organs, in order to analyse the results obtained on the whole animal. The effects seen in different species of animals have been compared in order to gain a wider basis on which to criticize the results.

The commercial extract known as pituitrin and the separated pressor and oxytocic principles, pitressin and pitocin, have all been used in different parts of the investigation.

The action of pituitary extracts on the heart and pulmonary circulation of the rabbit, the cat and the dog.

Rabbit.

Method. The experiments were made on animals anæsthetized with urethane (1·5 g. per kg.). The systemic arterial pressure was measured in the carotid with a mercury manometer. In order to measure the pressure in the auricles and in the pulmonary artery the chest was opened by the removal of the upper three or four ribs from their sternal ends. The pressure in the left auricle was measured by inserting a cannula either into the left auricle directly or through a pulmonary vein; in the right auricle by a long cannula inserted through the jugular vein, and in the pulmonary artery by Schafer's method. The pressure was recorded in millimetres of half-saturated sodium sulphate solution.

Results. The most striking effect of an intravenous injection of pituitrin in the rabbit is produced by its action on the heart. This is well shown by the volume curves obtained with the air oncometer. A small dose of 0·1 unit causes only a small diastolic dilatation by which the output of the heart is influenced probably only to a negligible degree. A somewhat larger dose of 0·3 unit leads to a more definite dilatation, the cardiac output per beat being definitely diminished for about half a minute, whilst the heart rate undergoes little change. Larger doses such as 0·5 unit profoundly weaken systole; the heart dilates and the rate of beat is diminished (Fig. 1). This is in conformity with many previous investigations [Tigerstedt and Airila, 1913; Börner, 1916; Müller, 1917; Wolfer, 1922].

Small doses of pituitrin—less than 0·3 unit—administered to the isolated rabbit's heart perfused with Ringer's solution by Langendorff's method, may diminish the coronary flow to one-half without influencing the heart beat, as recorded in the usual manner. These observations are in conformity with those of Anrep, who in the heart-lung preparation of the dog found that there is no relation between the

rate of the coronary flow and the action of the heart [Anrep and Bulatao, 1925; Anrep and Stacey, 1927–8]. It may be mentioned, however, that Bodo [1927] in experiments on the heart-lung preparation found that constriction of the coronary vessels was always accompanied by simultaneous dilatation of the ventricles; and Roessler [1930] thinks that the injurious effect of pitressin on the dog's heart is mainly due to constriction of the coronary vessels. After larger doses of pituitrin (0·5 unit and more) the constriction of the coronary vessels in the isolated

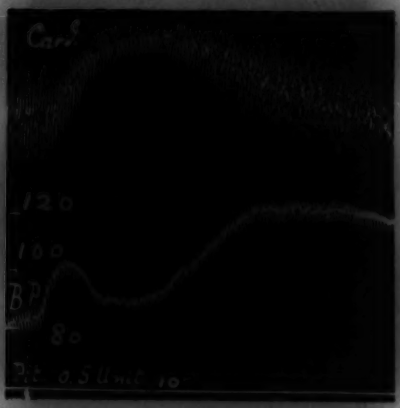

Fig. 1. Rabbit. Urethane. Cardiometer. Dilatation of the heart after 0·5 unit pituitrin. Time = 10 sec.

rabbit's heart occurs simultaneously with a decided decrease in frequency and a diminution in the size of the contractions. Thus the result of these experiments on the isolated rabbit's heart speaks in favour of a direct action of pituitrin on the heart muscle itself, besides its constrictor effect on the coronary vessels.

The reaction of the heart to pituitrin explains the peculiar effect on the carotid blood-pressure. Tracings of this show an initial rise followed by a distinct fall, which is then followed by a second and larger rise. The fall occurs simultaneously with the diastolic expansion and is clearly due to it (Fig. 1). The injured heart cannot overcome the increased systemic arteriolar resistance, and, as a result, the arterial pressure falls

to a certain extent. The heart, however, quickly recovers and the blood-pressure rises again.

The pressures in the left and in the right auricle fall when a small dose of pituitrin is injected. In one experiment a dose of 0·2 unit caused a fall of pressure of 10 mm. of half-saturated sodium sulphate solution in the right auricle and a fall of 12 mm. in the left auricle. These effects are probably due to the strong constriction of the coronary vessels, leading to a diminution of blood flow to the right auricle, so that the

Fig. 2. Rabbit. Urethane. Fall of pulmonary artery pressure (P.A.) after 2·0 units pituitrin. Time = 10 sec.

right ventricle sends a smaller amount of blood through the lungs into the left auricle. There is no back pressure from the ventricles, since the contractile power of the heart is not materially affected by these small doses of pituitrin.

If larger doses are injected, the heart beat is weakened and the back pressure of the dilated ventricles increases the pressures in the auricles.

The pressure in the pulmonary artery always falls after injections of pituitrin, no matter what the dose may be (Fig. 2), as also stated by Sharpey-Schafer and Macdonald [1926–7]. When the dose is small, this effect is probably due to the decrease in the flow through the coronary

vessels: the right heart receives less blood. After larger doses an increase of the pressure of the pulmonary artery might be expected, because the raised pressure in the left auricle augments the resistance in the pulmonary circulation. But these larger doses cause a profound weakening of the cardiac muscle. Thus the right ventricle does not only receive less blood by the constriction of the coronary vessels, but even sends out less blood than it receives. That again explains further the fact, which might appear paradoxical, that simultaneously with the fall of pressure in the pulmonary artery the pressure in the right auricle increases slightly.

The action of pituitrin on the pulmonary circulation of the rabbit depends therefore upon two main factors: (1) the depression of cardiac muscle, (2) the constriction of the coronary vessels.

Cat.

Method. The animals were anæsthetized with A.C.E. and urethane. In order to measure the pressure in the pulmonary artery, a cannula was tied into a branch on the hilus of the right upper or middle lobe. The lung volume was recorded by the Brodie-Dixon method with an air tambour connected to an oncometer enclosing the middle lobe of the right lung. The bronchus of this lobe was ligatured.

Results. From the experiments dealt with in the first part of this paper it is evident that in rabbits the action of pituitrin on the heart is of great importance for its effect on the pulmonary circulation. It therefore seems best, in describing the action of pituitrin on the cat's circulation, to begin by discussing its effects on the heart.

The investigations of previous workers [McCord, 1911; Biedl, 1913; Börner, 1916], most of which were published some time ago, show that the cat's heart is much more resistant to pituitrin than the rabbit's heart. I can confirm this. Fig. 3 shows the effect of 1·0 unit vasopressin on the isolated cat's heart. It can be seen clearly from it that the coronary flow is increased and the contractions of the heart enlarged: the heart is therefore stimulated. The same dose administered to the heart of a rabbit would lead to a considerable decrease of the coronary output and a reduction in the amplitude and frequency of the heart beat. Larger doses—3·0 units—either do not change the action of the cat's heart or cause a slight diminution in the contractions. The coronary vessels remain uninfluenced. The experiments with the cardiometer confirm these results. 1·0 unit of pituitrin or pitressin is almost without any effect, large doses produce only a small diastolic expansion. It can therefore be concluded, that small doses of pituitrin or pitressin—not

exceeding 1·0 unit—which would definitely damage the rabbit's heart, either leave the cat's heart entirely untouched or even lead to an improvement of its action, and that very large doses—up to 3·0 or 4·0 units—may slightly weaken the cat's heart, although the effect is still small compared with that seen in the rabbit's heart. The auricular pressures either remain unchanged or fall after small doses of pituitrin such as 0·5 or 1·0 unit. The fall of auricular pressure in the rabbit has already been attributed to a diminution in the blood flow to the right auricle, caused by constriction of the coronary vessels. In the isolated heart of the cat pituitrin has no such effect on the coronary vessels, and it is therefore probable that direct stimulation of the ventricles is the

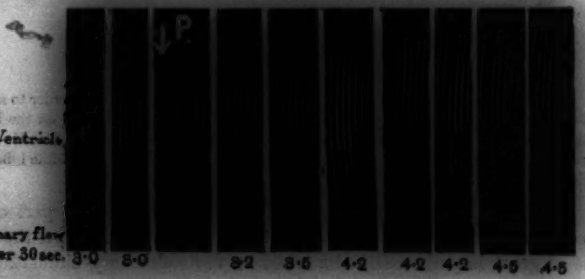

Fig. 3. Cat. Isolated heart (Langendorff's method) 1·0 unit pitressin (↓ *P.*) augments the coronary flow and stimulates the action of the heart. Each sector corresponds to 30 sec.

main cause of the small fall of auricular pressures which usually occurs. Large doses of pituitrin cause a rise of auricular pressure in the cat. No direct comparison has been made of the relative magnitude of the effect on the two auricles, but in a number of experiments in which the pressure in the right auricle was recorded, the effect of pituitrin was always larger than that seen in other experiments when the pressure in the left auricle was recorded. The rise in auricular pressure may be accounted for, as in the case of the rabbit, partly by weakening of the ventricles, but it will be seen later that there are two other factors which probably contribute to the rise of auricular pressure after large doses in the cat but not in the rabbit. In contrast to the effects seen in the rabbit the pressure in the pulmonary artery of the cat is invariably increased by the injection of pituitrin (Fig. 4). This effect might have

been due to vaso-constriction of the pulmonary vessels, but no such action could be demonstrated in the isolated and perfused lungs of the cat, although these vessels showed definite reactions to injections of small doses of adrenaline and histamine.

The fact that simultaneously with the rise of the pressure in the pulmonary artery the volume of the lung is augmented (Fig. 5), points to the suggestion that pitressin causes an increased blood supply to the lungs. This increase of blood flow to the cat's lungs may be due to two

Fig. 4.

Fig. 5.

Fig. 4. Cat. Urethane. Rise of pulmonary artery pressure (P.A.) after 2·0 units pitressin. Time = 10 sec.

Fig. 5. Cat. Urethane. Increase of lung volume (L.V.) and rise of pressure in the right auricle (R.A.) after 2·0 units pituitrin. Time = 10 sec.

causes: (1) Anrep and Bulatao [1925] found in investigations concerning the distribution of blood flow between the coronary and systemic vessels, that an increase in the systemic resistance produces an increased blood flow through the coronary vessels. Since the coronary vessels of the isolated cat's heart are not constricted by pitressin and may even be dilated, it is probable that in the whole animal the constriction of the systemic vessels leads to an actually increased coronary flow. As a result of this, more blood than normal will flow to the right heart and to the lungs; (2) Berry and Daly [1932], in perfusing the lungs simultaneously through the pulmonary artery and the posterior bronchial

artery, which latter takes its origin from the aorta, have shown that a rise of pressure in the bronchial artery causes a corresponding rise of pressure in the pulmonary artery and an increased output from the lung veins into the left auricle. Thus the augmented peripheral resistance, as caused by the injection of pitressin, might possibly increase the blood supply to the lungs through the bronchial arteries.

The action of pituitrin on the pulmonary circulation of the cat therefore depends upon the increase of blood supply to the lungs caused (1) by an active dilatation of the coronary vessels—after small doses, (2) by a passive increase in coronary flow which is due to the increase in systemic resistance—after large doses, and perhaps (3) by an increased blood flow through the bronchial circulatory system into the lungs, which also is caused by the augmented peripheral resistance.

Dog.

Method. The experiments on dogs were performed under chloralose anæsthesia (0·1 g. per kg. intravenously). The pressure in the pulmonary artery was measured in a side branch on the hilus of the right upper or middle lobe; the pressures in the left and right auricle by tying cannulæ direct into the appendices. The lung volume was recorded as described for the cat.

Results. If pituitrin or pitressin is injected intravenously into a dog, the rise in arterial blood-pressure is never so pronounced and immediate as in the rabbit and in the cat. Sometimes even a fall after an initial rise may be obtained. The carotid curve shows slowing of the pulse and irregularities in the action of the heart. In agreement with the results obtained by previous workers [Mautner and Pick, 1929; Kolls and Geiling, 1924; Roessler, 1930] it was found that pituitrin dilates the ventricles and constricts the coronary vessels: like the rabbit's heart, and in contrast to the heart of the cat, the dog's heart is very much injured by pituitrin.

The pressure in both auricles is raised, but the difference in the magnitude of the rise of pressure in the right and the left auricle is just the reverse of that found in the cat: while the pressure in the right auricle shows only a small rise, that in the left auricle is very considerably increased, sometimes tenfold or more (Fig. 6). Thus it appears that the left ventricle of the dog is injured by pitressin to a higher degree than the right ventricle, as also was concluded by Roessler from his experiments with the heart-lung preparation. The outflow from the lung veins into the left auricle is thus retarded, and one would expect that the pressure in the pulmonary artery would rise against the in-

creased resistance. But in spite of the increased pressure in the left auricle the pressure in the pulmonary artery always shows a pronounced fall (Figs. 7 and 8). It is obvious in so far as the heart is concerned that there are two factors tending to change the pulmonary arterial pressure in an opposite direction: (1) the back pressure from the left auricle tends to raise it, (2) the constriction of the coronary vessels, followed by a diminished blood flow to the right auricle, tends to lower it.

Fig. 6. Dog. Chloralose. Rise of pressure in the right and left auricle after 2·0 units pitressin. Time = 10 sec.

That after the injection of pitressin the blood supply to the lungs is actually diminished is proved by the decrease in lung volume (Fig. 9). The constriction of the coronary vessels, however, cannot be the only cause for the diminished blood flow to the lungs. From experiments on the heart-lung preparation I can confirm Roessler in that a second dose of pitressin has almost no effect on the coronary vessels. In one of my experiments for example the first injection of 3·0 units of pitressin diminished the flow through the coronary vessels from 58 c.c. per min. to 20 c.c. per min.; the same dose, injected 22 min. later, had practically no effect on the coronary vessels, producing only a diminution of flow

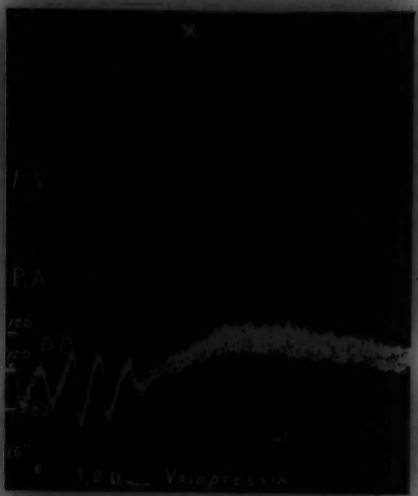

Fig. 7. Dog. Chloralose. Effect of 2·0 units of pitressin on the pressure in the pulmonary artery (P.A.) and the intestinal volume (I.V.). *X* = bellows lowered.

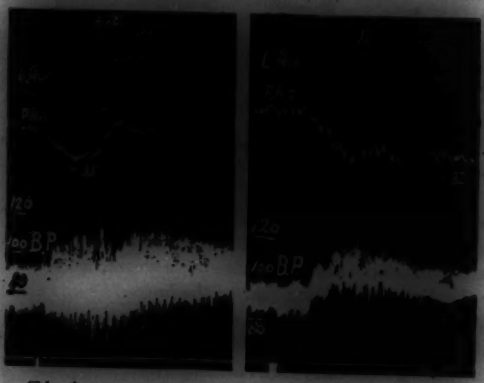

Fig. 8. Dog. Chloralose. (*a*) Injection of 1·0 unit pitressin: fall of pulmonary artery pressure (P.Art.); rise of left auricular pressure (L.Aur.). (*b*) 22 min. later; injection of 1·0 unit pitressin: fall of pulmonary artery pressure (P.Art.); slight fall of left auricular pressure (L.Aur.).

from 54 c.c. per min. to 50 c.c. per min. The coronary vessels therefore are refractory against a second injection of pitressin, the dilatation of the heart does not take place after the second dose and the pressure in the left auricle remains unchanged or even shows a slight fall (Fig. 8 *b*).

Fig. 9.

Fig. 10.

Fig. 9. Dog. Chloralose. Diminution of lung volume (L.Vol.) after 1·0 unit of pitressin. Time = 10 sec.

Fig. 10. Dog. Chloralose. Effect of 2·0 units of pitressin. Increase of intestinal volume and fall of portal pressure (P.P.). The rise of the systemic arterial pressure (B.P.) is only partially recorded.

In spite of this, the pressure of the pulmonary artery falls also after the second injection (Fig. 8 *b*). From this we can conclude that other parts of the circulatory system play an important part in the action of pitressin on the pulmonary circulation, and I think one is justified in assuming that the cause for the fall of pulmonary arterial pressure after a second injection of pitressin is the diminution in the return of blood

from the arterial side to the venous side of the vascular system. This view is supported by the fact that the pressure in the femoral and jugular vein falls if pitressin is injected.

It is therefore not surprising that in the heart-lung preparation the pressure in the pulmonary artery invariably rises after pitressin has been injected, because here the whole peripheral vascular system is ligatured off and the diminution of blood flow to the right heart, as caused by the constriction of the coronary vessels, is probably largely masked by the constant inflow from the venous reservoir. Thus the increased pressure in the left auricle is the only factor in determining the height of pressure in the pulmonary artery, which must therefore rise with a first, and remain unchanged with a second injection of pitressin, since heart and coronary vessels are now desensitized and the left auricular pressure does not rise a second time.

The action of pituitrin on the pulmonary circulation of the dog therefore depends upon the diminished blood supply to the lungs as caused (1) after the first injection by constriction of the coronary and systemic vessels, (2) after the second injection by constriction of the systemic vessels only.

The action of pituitary extracts on the mesenteric and portal circulation of the cat and the dog.

In confirmation of the results obtained by previous investigators [Miura, 1925; Clark, 1928], it was found that the portal pressure in cats and dogs, as measured in the splenic vein, shows a distinct fall if pituitrin or pitressin is injected into the general circulation. This corresponds with the observation of Mautner and Pick [1929], that the volume of the liver as measured in the living animal is diminished.

The fall of portal pressure and the diminution in liver volume is usually attributed to the fact that pituitrin constricts the splanchnic vessels and thus diminishes the blood supply to the portal system. This view is supported by the early observations of Oliver and Schafer [1895], that in the cat the intestinal volume is diminished after an injection of pituitary extract. Thus it seems that in the cat a diminished blood supply to the portal system, caused by constriction of the splanchnic vessels, might be sufficient to explain the fall in portal pressure and the diminution in liver volume. In the dog, however, this explanation does not apply, since in the dog (Fig. 10) simultaneously with the fall in portal pressure the intestinal volume after a short decrease is augmented.

The explanation of the fact, that in the dog the liver shrinks and the pressure in the portal vein falls, although the blood flow to the splanchnic area is increased, might be found either in that pituitrin constricts the mesenteric veins, thus leading to an accumulation of blood in the intestinal vessels and a diminution of blood flow into the portal vein and to the liver, or in that it has a dilator effect on the outflow side of the portal system, *i.e.* on the hepatic veins, thus diminishing the resistance, against which the blood from the portal system flows into the vena cava.

Both possibilities were examined by performing perfusion experiments (*a*) on isolated mesenteric vessels and (*b*) on the isolated liver.

(*a*) *Perfusion of isolated mesenteric vessels of cats and dogs.*

Method. The animals were anæsthetized with ether-chloroform and bled from the carotid. The blood was defibrinated and the perfusion apparatus filled. Cannulæ were then tied in the superior mesenteric artery and the splenic vein, and the whole small intestine separated and divided between two ligatures. The blood in the mesenteric vessels was washed out by perfusion through the splenic vein with hot Ringer. Then the intestine was resected at the insertion of the mesentery, so as to perfuse *either* the isolated mesenteric arteries *or* the veins in the reversed direction, the blood escaping from the cut arterioles and venules, as the case might be, without passing through capillaries [Dale and Richards, 1919]. The artery was perfused by means of the Dale-Schuster pump, the vein by hydrostatic pressure (in other experiments also the artery perfusion was performed by hydrostatic pressure). By means of clips it was possible to perfuse separately either the artery or the vein. The outflow was recorded by the method described by Gaddum [1929].

Results. The mesenteric arteries both of cats and dogs were shown in this way to be constricted by pitressin. This effect of pitressin (Fig. 11 *b*) like its effect on the general blood-pressure was definitely more prolonged than the corresponding constrictor effect of adrenaline (Fig. 11 *a*) on the same preparation.

The action of pitressin on the mesenteric veins is quite different from that of adrenaline. In Fig. 12 *a* it will be seen that a small dose of adrenaline produced in cats and dogs marked constriction of the mesenteric veins similar to its effect on the mesenteric arteries. The only effect seen after injection of pitressin (Fig. 12 *b*) was a small dilator effect.

The result then of the experiments with pitressin on the isolated mesenteric vessels is that, both in cats and dogs, it constricts the arteries and has, if any, a weak dilator effect on the veins.

(*b*) *Perfusion of the isolated livers of cats and dogs.*

Method. Livers isolated from cats and dogs were perfused simultaneously through the hepatic artery and the portal vein with the defibrinated blood of the same species. In the case of the cat the blood from three cats was used. The perfusion through the hepatic

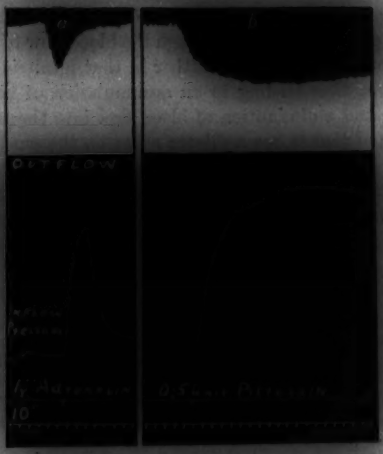

Fig. 11. Dog. Perfusion of isolated mesenteric arteries. (*a*) Injection of 1γ of adrenaline. (*b*) Injection of 0·5 unit of pitressin.

Fig. 12. Dog. Perfusion of isolated mesenteric veins. (*a*) Injection of 5γ of adrenaline. (*b*) Injection of 2·0 units of pitressin.

artery was performed by means of the Dale-Schuster pump, the portal vein was perfused from a blood reservoir by hydrostatic pressure. The records of the tracings beginning with the top one show: (1) outflow from the vena cava measured with the method described by Gaddum, (2) liver volume by plethysmograph, kept at constant temperature and connected to large bellows, (3) portal pressure recorded by means of a water manometer and a Brodie bellows, (4) arterial pressure in mm. Hg. Full details of the method are given in a paper by Bauer, Dale, Poulsson and Richards [1932].

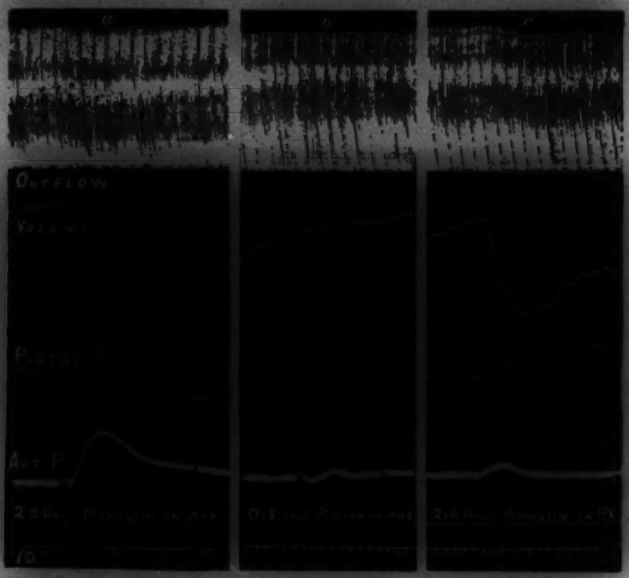

Fig. 13. Perfusion of cat's liver. (a) Arterial injection of 2·0 units of pitressin. (b) Arterial injection of 0·5 unit of pitocin. (c) Portal injection of 2·0 units of pitressin.

Results. Lampe and Méhes [1926] have performed experiments with pituitary extracts on the isolated liver of cats and dogs, Clark [1928] on the isolated dog's liver. In both cases the liver was perfused only through the portal vein, and pituitrin produced diminution of the liver volume and of the outflow from the vena cava, both of which effects were ascribed to constriction of the portal vessels. Adrenaline had a similar but stronger effect.

Cat's liver. The effects of pitressin and pitocin on the cat's liver are shown in Fig. 13. When pitressin is injected into the hepatic artery it produces a rise in the arterial pressure, a fall of portal pressure and a fall of volume (Fig. 13 *a*). These effects are probably due to the constriction of the branches of the artery, the fall of portal pressure being a direct mechanical result of the decrease in the rate of flow through the artery. The blood supply to the liver through the portal vein is thus somewhat increased and compensates almost completely for the decrease in arterial flow, so that only a just perceptible decrease in the total flow results, as shown by the slightly diminished outflow. When pitressin is injected into the portal vein, the portal pressure rises, presumably owing to constriction of the portal vessels (Fig. 13 *c*).

Pitocin has little or no effect on the circulation of the isolated liver of the cat (Fig. 13 *b*).

The fall in portal pressure and liver volume, seen after pituitrin in experiments on the whole cat, can thus be attributed to two causes: (1) selective constriction of the mesenteric arteries, leading to a diminution of the amount of blood flowing into the portal system, (2) constriction of the hepatic artery, leading to a decrease in the amount of blood reaching the liver by this route.

Dog's liver. As in the liver of the cat, the intra-arterial injection of pitressin produces a rise of arterial pressure (Fig. 14 *a*), but the accompanying fall of portal pressure and liver volume is more marked than in the cat, and the most striking difference between the two animals lies in the marked increase in the venous outflow which pitressin causes in the dog but not in the cat. The combination of these effects—fall in portal pressure and liver volume, and increase of outflow—can only be due to the relaxation of efferent vessels.

It is known that the dog's liver in contrast to that of the cat has a venous sluice mechanism [Mautner and Pick, 1923]. The efferent hepatic veins are constricted by small doses of histamine and, as has been found by Bauer, Dale, Poulsson and Richards [1932] and by Grab, Janssen and Rein [1929 *a*, *b*] relaxed by adrenaline. This sluice is apparently located near the openings of the hepatic veins into the vena cava, since extirpation of this region abolishes the effect. The corresponding effect of pituitary extract in my experiments similarly was found to be abolished by extirpation of the openings of the hepatic veins.

The present experiments thus show that the action of pituitary extracts on the vessels of the dog's liver is due to the relaxation of the efferent hepatic veins, the sluice veins of the dog's liver, and thus provide

an explanation for the fact that in the whole animal after an injection of pituitrin or pitressin portal pressure and liver volume fall, although the blood flow to the splanchnic area is increased (augmentation of intestinal volume). The pituitrin reaches the liver through the hepatic

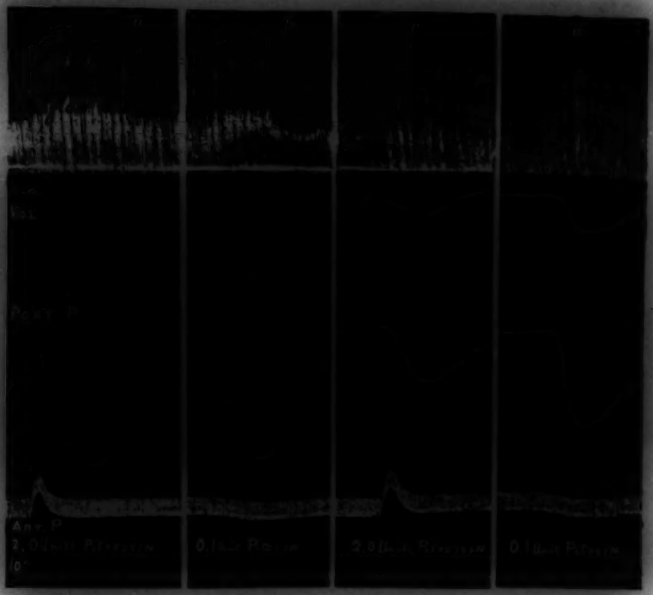

Fig. 14. Perfusion of dog's liver. (*a*) Arterial injection of 2·0 units of pitressin. (*b*) Arterial injection of 0·1 unit of pitocin. (*c*) Arterial injection of 2·0 units of pitressin. (*d*) Arterial injection of 0·1 unit of pitocin.

artery and relaxes the venous sluice, before the augmented blood flow to the splanchnic area can produce any rise in portal pressure. This relaxation is so marked that the total effect produced is a pure fall of portal pressure.

In the course of these investigations it was found that pituitrin, the complete extract of the posterior lobe, caused an even more pronounced increase of outflow from the dog's liver than pitressin, and the possi-

bility arose that this effect might be due not to the pressor principle itself, but to the oxytocic principle, which is always present in small quantities in the pitressin but which is of course present in larger quantities in the pituitrin. To test this point a preparation of the separated oxytocic principle, pitocin, was first compared with pitressin for its action on the guinea-pig's uterus. It was found that 20 units of pitressin, tested in this way, were equivalent to 1 unit of pitocin. If the suggestion mentioned above, that the action of pitressin on the venous outflow in the dog's liver is due to its content of oxytocic principle, were correct, pitocin should have the same effect as pitressin when given in 1/20th of the dose. That this is indeed the case is shown in Fig. 14: 2 units of pitressin have approximately the same effect as 0·1 unit of pitocin. These results show that the effect of pituitary extracts on the venous outflow of the dog's liver is due to some substance which is present in much higher proportions in pitocin than in pitressin, and there is no reason to doubt that this substance is the oxytocic principle itself.

Fig. 15. Fowl. Urethane. Blood-pressure. Comparison between pitressin and pitocin.

Note on the action of posterior pituitary extracts on the blood-pressure of the fowl.

Support of the view that the dilator action of pituitary extracts (as seen on the mesenteric veins and the efferent hepatic veins of the dog's liver) is due to their content of oxytocic principle can be found in the result of some experiments on fowls. It is known that the blood-pressure of the bird, in contrast to that of the mammal, falls after the injection of posterior pituitary extracts [Paton and Watson, 1912; Hogben, 1925]. Gaddum [1928] and Morash and Gibbs [1929] found that this effect could be obtained with smaller doses of "oxytocin" than of "vasopressin."

Fig. 15 shows records from an experiment, in which a quantitative comparison was made between the action of pitressin and pitocin on

the blood-pressure of the fowl. From Fig. 15 *a* and *c* it can be seen that a twenty times smaller dose of pitocin (0·1 unit) than of pitressin (2 units) has approximately the same effect, and this effect can be obtained several times in the same experiment. Thus it seems conclusive that the fall of blood-pressure, as observed in birds after the injection of pituitary extracts, is caused by the oxytocic substance.

In Fig. 15 *b* a very small dose of pitressin (0·1 unit) has, after a slight dilator, a small pressor effect. The amount of oxytocic substance contained in the small dose of 0·1 unit of pitressin is apparently not big enough to overcome the pressor action which the entirely pure pressor principle of the posterior lobe probably would have also in the bird. Finally, Fig. 15 *d* shows the pronounced and very long-lasting dilator effect of a somewhat larger dose of pitocin (2 units).

Discussion.

A point needing a little further discussion is the question of the significance of the relaxation of the hepatic veins, as seen after the injection of pituitary extracts in the isolated liver of the dog, for the portal and the pulmonary circulation in the whole animal. It has been pointed out that the fall of portal pressure, which occurs in the whole animal in spite of the increased blood flow to the splanchnic area (increased intestinal volume), could be explained by assuming that the opening of the venous sluice in the dog's liver diminishes the resistance, against which the blood from the portal system flows into the vena cava. If in the whole animal, as in the isolated liver, the rate of outflow from the hepatic veins is thus augmented, this effect by itself would increase the flow of blood to the heart and lungs. The fall of pressure in the pulmonary artery and the diminution of lung volume, however, prove that, in fact, the total flow of blood to the lungs is diminished.

The blood flowing into the vena cava comes from two main sources: (1) from the splanchnic vessels through the portal vein, liver and hepatic veins, (2) from the limbs, body wall and the somatic area generally. In experiments in which limb and intestinal volumes were measured simultaneously it was found that the limb volume was diminished at the same time as the intestinal volume was augmented. The limb vessels of the dog, therefore, seem to be more sensitive to the constrictor action of pituitrin than the splanchnic vessels. Under these conditions the return of blood from the somatic area to the heart and lungs is diminished, the blood flow to the splanchnic area, in which the constrictor effect is

less powerful, undergoing a passive augmentation. The discharge of blood from the splanchnic area by the hepatic veins into the cava cannot, however, be increased to the same extent as that from the somatic area is diminished, since the total inflow to the right heart becomes less, causing a fall of the pulmonary arterial pressure. There must accordingly be some accumulation of blood in the splanchnic area, and we have seen that this occurs in the intestine, the volume of which shows a secondary increase. Owing to weakening of the resistance to hepatic outflow, the liver loses volume and the pressure falls in the portal vein. Since, as shown in perfusion experiments, the tone of mesenteric venules is not increased, but, on the contrary, somewhat diminished, the combined effect of swelling of the intestine and fall of portal pressure is still inadequately explained. It seems necessary to postulate a loss of tone in the intestinal capillaries to account for it.

Summary.

The action of extracts of the posterior lobe of the pituitary gland on different parts of the circulation has been studied.

1. *Pulmonary circulation and heart.* Pituitary extracts have no action on the lung vessels. Either the complete extract (pituitrin) or the separated pressor fraction (pitressin) produces a rise of pressure in the pulmonary artery of the cat, which is secondary to an increase of coronary flow; they produce a fall of pulmonary arterial pressure in the rabbit and in the dog, which is due to a diminution of coronary flow. An additional cause of this fall in the dog is the accumulation of blood in the splanchnic area. The reason for the rise of pressure in the pulmonary artery in the heart-lung preparation of the dog is discussed.

2. *Portal circulation.* Pituitary extracts cause a fall of pressure in the portal vein both of the cat and the dog. In the cat this is due to selective constriction of the mesenteric arteries and to constriction of the hepatic artery. In the dog it is mainly caused by dilatation of the hepatic veins (opening of the venous "sluice" of the dog's liver). This dilator effect on the hepatic veins, produced by pituitrin and pitressin, is due to their content of oxytocic substance. (The fall of blood-pressure in the fowl after the injection of pituitary extracts is also due to the oxytocic principle.)

I wish to thank both the late Prof. W. E. Dixon and Sir Henry Dale, whose advice and criticism have been invaluable at different stages of this work.

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THE RESTING HEAT PRODUCTION OF NERVE.

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THE rate at which heat is liberated by a muscle at rest on a thermopile can be measured directly as a deflection on a galvanometer scale, provided that certain precautions are taken [see Hill, 1928 *a*]. These are (*a*) the use of instruments so well insulated that electrical leaks and disturbances do not occur; (*b*) the employment of a constant temperature bath of sufficient accuracy; (*c*) the avoidance of a vapour pressure difference between the muscle and the gas in the chamber. If this difference be not avoided [Hill and Kupalov, 1930] large errors may occur due to evaporation of, or condensation on, the muscle.

Similar methods can be used to measure the resting heat production of nerve. In the present work a differential nerve thermopile described by Downing and Hill [1929] was used together with a Downing moving magnet galvanometer (50 ohms resistance, 1 mm. $= 4 \times 10^{-10}$ amp. about). For the measurement of resting heat a differential thermopile has great advantages, since slight fluctuations which cannot be avoided in any bath, however well controlled, then produce less instability. A gas-regulated thermostat bath was employed, constant to about 0·002° C. under good conditions [see Hill, 1930]. To avoid a vapour pressure difference it was necessary to soak the nerve in Ringer's solution for a long time before readings were made. For this purpose the nerves must be firmly fixed to the thermopile so as not to be washed away by the fluid in the chamber.

In all experiments four sciatic nerves of Hungarian *Rana esc.* were used. After careful dissection they were mounted on the thermopile, each nerve running twice along the thermopile face, being wound round the two warming electrodes, one at each end. Ringer's solution was placed in the chamber (phosphate 10 mg. per 100 c.c., *p*H 7·2), the apparatus was placed in the bath and oxygen was bubbled through the solution to assist in temperature equalization, and to keep the nerve well oxygenated.

Nerves appear to take a long time to reach vapour pressure equilibrium with a surrounding solution, so that dissection usually occurred the evening before the experiment. The nerves therefore were soaked in Ringer's fluid all night—about 12 hours. When observations were to begin, the solution was removed and replaced by oxygen and in about 20 min. the first reading of the resting heat rate in oxygen was made. To obtain a good average the readings were continued for half an hour. The oxygen was then replaced by nitrogen, which had been purified by passing over hot copper and moistened by bubbling through water. This passed through the chamber rapidly for the first 4 min. and then more slowly. The slow passage of nitrogen continued throughout the period of asphyxia. This lasted for 3–5 hours; oxygen was then readmitted slowly so as not to cause a disturbance, and readings of the heat production during recovery from asphyxia were made.

At the end calibration was carried out with condenser discharges and a commutator, as recently described [Hill, 1931].

The temperature was about 20° C. throughout. Owing to the sensitivity of the arrangements used, variations of temperature in the bath, too small or too rapid to be read with a Beckmann thermometer graduated in 1/500° C., caused fluctuations in the readings of the resting heat rate, serious individually but capable of being avoided by taking an average over several minutes.

THE RESTING HEAT RATE OF NERVES IN OXYGEN.

Readings were taken every half minute for half an hour, and then the mean deflection for every 5 min. was calculated.

The following examples illustrate the procedure and the accuracy of the results.

Exp. of Feb. 12, 1932. Nerves dissected Feb. 11 at 7 p.m.; mounted on thermopile at 7.15 p.m. and put in Ringer's solution in bath at constant temperature. Feb. 12 at 10.35 a.m., Ringer's solution replaced by oxygen; at 10.55 a.m., readings begun.

Successive readings of the rate of heat production, each the mean for 5 min., were as follows, expressed in g. cm. of energy per g. of nerve per minute: 154, 152, 161, 148, 168, 157, 157, 151; mean 155.

Exp. of Feb. 15, 1932. Nerves dissected and put in Ringer's solution on thermopile in bath, Feb. 14 at 7 p.m. Next morning at 11.10 a.m. solution replaced by oxygen; at 11.53 a.m. readings begun. Mean for 30 min.: 224 g. cm. of energy per g. of nerve per minute. Mean variation of the 5 min. averages from the mean value: 2 p.c.

The results of eight experiments of the resting heat rate of nerves in oxygen are as follows: the total weight of the four nerves employed was

about 180 mg.: 155, 116, 172, 134, 186, 155, 224, 224; mean 176 g. cm. of energy per g. of nerve per min., or $4{\cdot}14 \times 10^{-3}$ cal. per g. per min.

The mean value agrees closely with that for muscle at the same temperature. Hill [1928 *a*, p. 139] gives 160 g. cm. per g. per min. as a "reasonable minimum" for 20° C. For nerves at rest at 14° C. Gerard [1927] found a mean oxygen consumption of $2{\cdot}7 \times 10^{-4}$ c.c. per g. per min. Extrapolating by means of a temperature coefficient of 2·5 for 10° C., the value at 20° C. should be $4{\cdot}7 \times 10^{-4}$ c.c. per g. per min. Taking 5 cal. as equivalent to 1 c.c. of oxygen, Gerard's extrapolated value is equivalent to $2{\cdot}35 \times 10^{-3}$ cal. per g. per min., or about 100 g. cm. per g. per min. According to Fenn [1927] at 22° C. the resting oxygen consumption of frogs' sciatic nerve is $1{\cdot}23 \times 10^{-3}$ c.c. per g. per min., which, extrapolated to 20° C. by the same temperature coefficient, would be $1{\cdot}03 \times 10^{-3}$ c.c. per g. per min. This is equivalent to $5{\cdot}15 \times 10^{-3}$ cal. or 220 g. cm. per g. per min. The mean value found in the present experiments lies almost exactly half-way between those calculated from the oxygen measurements of Gerard and Fenn.

Anaerobic resting heat production.

When oxygen was replaced by nitrogen, within 15 min. the rate of heat production began slowly to decrease. After 40 min. it had fallen to 75 p.c. of its initial value; at 1½ hours to 50 p.c. and at 2½ to 3½ hours to 20–25 p.c. After reaching 20–25 p.c. of its original level the heat rate ceased to fall and became constant apparently indefinitely.

Fig. 1 shows the behaviour of the heat rate during anaerobiosis. It was not possible to determine how long it remained at the minimum level. Four to four and a half hours after removing the Ringer's solution there might occur a slow increase of heat rate overshadowing the previous fall. This may have been due either to a decrease of vapour pressure caused by anaerobic breakdown or to some genuine heat production resulting from the injurious effect of long oxygen want. Observation showed at any rate that no significant increase occurred for 30–40 min. after the heat rate had fallen to one-quarter or one-fifth of its original level.

Table I gives in g. cm. per g. per min. the minimum heat rate of a resting nerve deprived of oxygen for 2–3 hours. For comparison the initial heat rate in oxygen also is given, and in the last column the former expressed as a percentage of the latter.

In order to decide to what degree the failure of the resting heat production depends upon the amount of oxygen initially present in the

TABLE I.

Date	Initially in O_2	Means in successive 5 min. in N_2	Mean of all in N_2	In N_2 as p.c. of initial in O_2
20. i. 32	172	50·0, 47·6, 49·9, 52·0	49·9	28·3
30. i. 32	134	—	29·1	21·7
2. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0
12. ii. 32	186	47·3, 47·0, 47·6, 47·8	47·3	25·0
15. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0

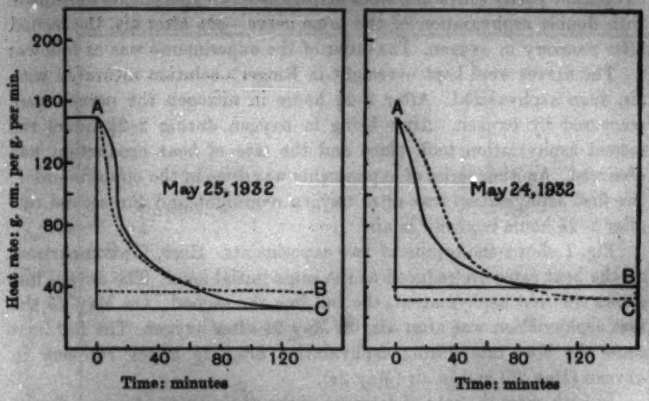

Fig. 1. Resting heat production of nerve in nitrogen at 21° C_x after oxygen or air.
Left: ..... *AB*, First asphyxiation after air.
—— *AC*, second asphyxiation after recovery in O_2.
Right: ..... *AC*, first asphyxiation after O_2.
—— *AB*, second asphyxiation after recovery in air.

environment, or in the nerve itself, four experiments were done in air instead of oxygen. All the conditions were the same, but instead of oxygen, air was bubbled through the Ringer's solution during the night. Then the resting heat rate, first in air and after that during asphyxiation, was read.

The results are given in Table II.

TABLE II.

Date	Heat initially in air g. cm./g. min.	Mean minimum value in N_2 g. cm./g. min.	In N_2 as p.c. of initial in air
18. v. 32	179	37·6	19·3
20. v. 32	238	50·0	23·5
23. v. 32	180	37·4	20·7
25. v. 32	198	51·1	25·7
Mean	198·8	45·5	22·9

about 180 mg.: 155, 116, 172, 134, 186, 155, 224, 224; mean 176 g. cm. of energy per g. of nerve per min., or $4{\cdot}14 \times 10^{-3}$ cal. per g. per min.

The mean value agrees closely with that for muscle at the same temperature. Hill [1928 *a*, p. 139] gives 160 g. cm. per g. per min. as a "reasonable minimum" for 20° C. For nerves at rest at 14° C. Gerard [1927] found a mean oxygen consumption of $2{\cdot}7 \times 10^{-4}$ c.c. per g. per min. Extrapolating by means of a temperature coefficient of 2·5 for 10° C., the value at 20° C. should be $4{\cdot}7 \times 10^{-4}$ c.c. per g. per min. Taking 5 cal. as equivalent to 1 c.c. of oxygen, Gerard's extrapolated value is equivalent to $2{\cdot}35 \times 10^{-3}$ cal. per g. per min., or about 100 g. cm. per g. per min. According to Fenn [1927] at 22° C. the resting oxygen consumption of frogs' sciatic nerve is $1{\cdot}23 \times 10^{-3}$ c.c. per g. per min., which, extrapolated to 20° C. by the same temperature coefficient, would be $1{\cdot}03 \times 10^{-3}$ c.c. per g. per min. This is equivalent to $5{\cdot}15 \times 10^{-3}$ cal. or 220 g. cm. per g. per min. The mean value found in the present experiments lies almost exactly half-way between those calculated from the oxygen measurements of Gerard and Fenn.

Anaerobic resting heat production.

When oxygen was replaced by nitrogen, within 15 min. the rate of heat production began slowly to decrease. After 40 min. it had fallen to 75 p.c. of its initial value; at 1½ hours to 50 p.c. and at 2½ to 3½ hours to 20–25 p.c. After reaching 20–25 p.c. of its original level the heat rate ceased to fall and became constant apparently indefinitely.

Fig. 1 shows the behaviour of the heat rate during anaerobiosis. It was not possible to determine how long it remained at the minimum level. Four to four and a half hours after removing the Ringer's solution there might occur a slow increase of heat rate overshadowing the previous fall. This may have been due either to a decrease of vapour pressure caused by anaerobic breakdown or to some genuine heat production resulting from the injurious effect of long oxygen want. Observation showed at any rate that no significant increase occurred for 30–40 min. after the heat rate had fallen to one-quarter or one-fifth of its original level.

Table I gives in g. cm. per g. per min. the minimum heat rate of a resting nerve deprived of oxygen for 2–3 hours. For comparison the initial heat rate in oxygen also is given, and in the last column the former expressed as a percentage of the latter.

In order to decide to what degree the failure of the resting heat production depends upon the amount of oxygen initially present in the

TABLE I.

Date	Initially in O_2	Means in successive 5 min. in N_2	Mean of all in N_2	In N_2 as p.c. of initial in O_2
29. i. 32	172	50·0, 47·6, 49·9, 52·0	49·9	29·3
30. i. 32	134	—	29·1	21·7
2. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0
12. ii. 32	186	47·3, 47·0, 47·6, 47·8	47·3	25·0
15. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0

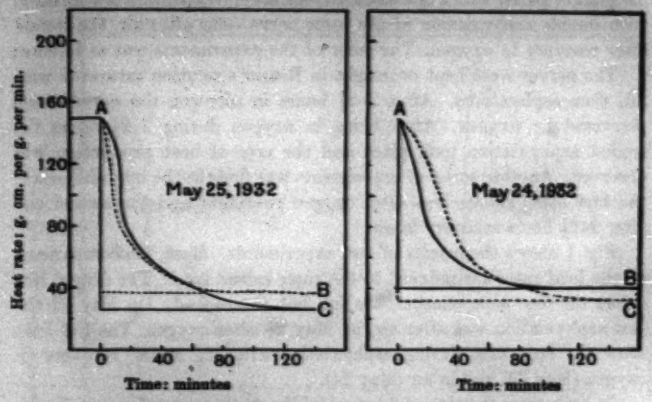

Fig. 1. Resting heat production of nerve in nitrogen at 21° C. after oxygen or air.
Left: ..... *AB*, First asphyxiation after air.
—— *AC*, second asphyxiation after recovery in O_2.
Right: ..... *AC*, first asphyxiation after O_2.
—— *AB*, second asphyxiation after recovery in air.

environment, or in the nerve itself, four experiments were done in air instead of oxygen. All the conditions were the same, but instead of oxygen, air was bubbled through the Ringer's solution during the night. Then the resting heat rate, first in air and after that during asphyxiation, was read.

The results are given in Table II.

TABLE II.

Date	Heat initially in air g. cm./g. min.	Mean minimum value in N_2 g. cm./g. min.	In N_2 as p.c. of initial in air
18. v. 32	179	37·6	19·3
20. v. 32	236	39·0	23·5
23. v. 32	189	37·4	29·7
25. v. 32	198	41·1	29·7
Mean	199·6	43·5	23·9

Comparing Tables I and II, we see that the resting heat rate and the minimum steady level reached during asphyxia are the same in both cases; the time, however, for reaching the minimum level for the nerve initially in air is less than for that initially in oxygen. If the latter reaches this level in about 2½ hours, the former reaches it in about 1½ hours.

Since the experiments in air were done considerably later than those in oxygen and partly with a new stock of frogs, several experiments were made with double asphyxiation of the same nerve—one after air, the second after recovery in oxygen. The order of the experiments was as follows:

The nerves were kept overnight in Ringer's solution saturated with air, then asphyxiated. After 2–2½ hours in nitrogen the nerves were recovered by oxygen. After being in oxygen during 2–2½ hours the second asphyxiation took place and the rate of heat production was observed. Another series of experiments was done in the opposite order: the first asphyxiation was after oxygen overnight and the second one after 2–2½ hours recovery in air.

Fig. 1 shows the results of two experiments. Here, for comparison, all the heat rates are reduced to the same initial level. The dotted line shows the first asphyxiation, the full line the second. On May 25 the first asphyxiation was after air, on May 24 after oxygen. The full lines show the heat rate during asphyxiation after 2½ hours' recovery in oxygen (May 25) and in air (May 24).

In both cases the rate of decrease of the resting rate of nerves during asphyxiation is slightly greater for nerves initially in air, whether this asphyxiation was the first or the second of an experiment.

The mean value of the anaerobic heat rate was 42·4 g. cm. per g. per min. This is only two-thirds of the value (65) given by Hill [1928*a*, p. 141] for muscle in nitrogen at 20° C. Hill's value was confirmed in two experiments made specially on muscle, which gave 72·5, 55·9, mean 64·2. These values were respectively 45 p.c. and 56 p.c., mean 50 p.c., of the heat rate initially in oxygen. The usual ratio of 1 to 2 was found, in striking contrast with the case of nerve, where the ratio is 1 to 4 or 1 to 5.

There is a further difference between muscle and nerve. In muscle the fall of resting heat rate is complete in 30–60 min. after oxygen is replaced by nitrogen; in nerve the diminishing heat rate continues for not less than 2½ hours. Moreover in muscle, as is well known, in recovery after asphyxia the amount of excess heat is about equal to the amount of heat missed during the period of oxygen want. In nerve, as we shall see, the excess is only a small part of the heat missed.

The recovery heat production after asphyxia.

Fig. 2 shows the behaviour of asphyxiated nerve when oxygen is readmitted. The heat rate begins to increase immediately; in 5 min. it reaches the original level and in 10–15 min. it may be 50–60 p.c. above the original level. During the next 15 min. a rapid decrease occurs which

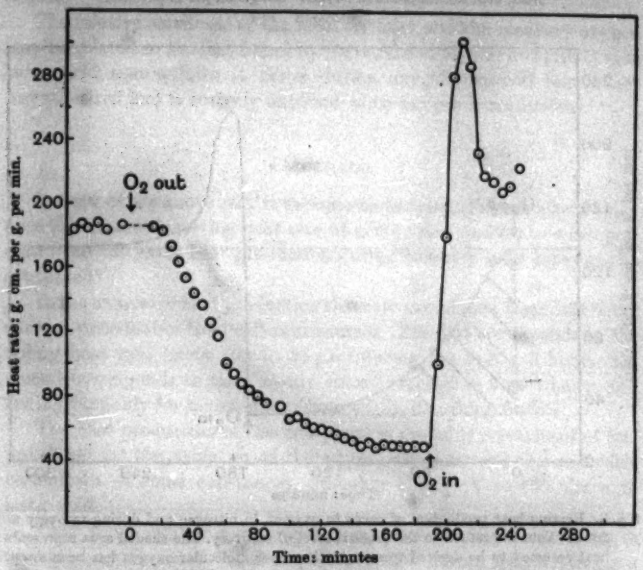

Fig. 2. Rate of heat production of nerve in oxygen and in nitrogen at 20° C. Oxygen removed at the moment shown by the first arrow and reintroduced at the moment shown by the second arrow.

afterwards becomes slower until a steady level is reached. In one experiment only (Fig. 3) was the final level after recovery from asphyxia the same as the original level in oxygen. Generally after decreasing to 115–120 p.c. of its level, the heat rate began slowly to rise again, due to some unknown cause, perhaps to a harmful effect of long oxygen want.

The maximum heat rate observed during recovery had a mean value of about 7×10^{-3} cal. per g. per min., which is about seven times the minimum heat rate in nitrogen.

The most striking thing about these results is the smallness of the recovery heat in comparison with the heat missed in nitrogen. Let us calculate the amounts.

Exp. of Jan. 7, 1932.

Mean heat rate of nerve in oxygen 155 g. cm. per g. per min.
" " nitrogen 79·4 "

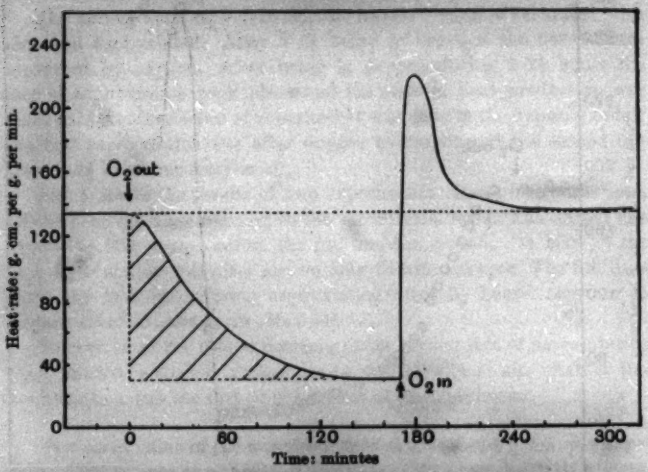

Fig. 3. Resting heat production of nerve in oxygen, in nitrogen and during recovery at 20° C. Note the return to the original level of recovery. The shaded area represents heat assumed to be derived from oxidation after molecular oxygen has been swept away.

Total heat in nitrogen during the whole period of asphyxiation (3 hours 12 min.) = 15230 g. cm. per g.

Total resting heat in oxygen for same period = 29800 g. cm. per g.
Heat (missed) in nitrogen = 14570 g. cm. per g.

Recovery heat after oxygen readmitted:

Mean heat rate 2 hours after	174 g. cm. per g. per min.
Total heat during recovery (2 hours)	20800 g. cm. per g.
Excess heat for this period	20800 – 18600 = 2200 g. cm. per g.

Thus we have:

Heat missed in nitrogen	14570 g. cm. per g.
Excess heat during recovery	2200 g. cm. per g

It is interesting to compare the excess heat of recovery found here with the excess oxygen found by Fenn [1930]. Fenn found that after a period of oxygen want nerves consumed an extra amount of oxygen which, however, was less than one-quarter and frequently less than one-tenth of the oxygen missed during the anaerobic period. The phenomenon found here is analogous to that found by Fenn. The extra oxygen and the extra heat when oxygen is admitted after asphyxia are presumably related.

The relative smallness of the recovery heat and the recovery oxygen may be related to the fact found by Gerard and Meyerhof [1927] that lactic acid accumulates in nerve during oxygen deprival but is not resynthesized and is scarcely oxidized when oxygen is readmitted.

Discussion.

In view of the above results two questions arise: (1) from what source does the anaerobic resting heat rate of nerve arise, and (2) to what processes can the extra heat production during recovery from asphyxia be attributed?

In the anaerobic heat production there are two phases, their heat being derived presumably from different sources. The first corresponds to the falling heat rate (from 100 to 25 p.c.) during the first 2–3 hours, the second corresponds to the "steady state" reached in 2 or 3 hours and lasting probably for many, apart from slight disturbing factors.

The heat production of the first phase is probably a resultant of two processes: (*a*) the using up of the oxygen store and (*b*) the anaerobic breakdown of some substances, probably chiefly of carbohydrate to lactic acid.

That some oxidative processes take place during anaerobiosis is indicated by Gerard's discovery [1927] that during long asphyxia a nerve produces (not merely gives off) about 10 p.c. as much carbon dioxide as during the same time in oxygen. The question is what kind of oxygen it can be—whether a true "oxygen store," the existence of which in the nerve is suggested by various authors, or whether molecular oxygen initially dissolved in the nerve tissue, which does not escape by diffusion.

Let us calculate the amount of heat production due to this "oxidative" process. We can do it assuming that during asphyxia all the heat above the minimum steady level (equal to 20–25 p.c. of the initial level) is derived from this source. In Fig. 3 the shaded area represents the heat assumed to be so derived.

In the experiments of Jan. 7 and Jan. 30 the "oxidative" heat calculated in this way during asphyxia had the values 4240 and 2430 g. cm. per g., and in all the experiments performed the mean value was 3120 g. cm. per g. This would be enough to last at the full resting heat rate in oxygen for about 20 min.

The total amount of oxygen corresponding to it can be calculated, assuming again that 5 cal. are equivalent to 1 c.c. of oxygen: 3120 g. cm. per g. is then equivalent to 0·0146 c.c. of oxygen per g. This is quite a small amount when we remember that water saturated with oxygen at 20° contains 0·031 c.c. of oxygen per g., so that nerves containing 80 p.c. of water would hold 0·025 c.c. of oxygen per g. in simple solution.

In what way will this "oxidative" heat be changed in the case of nerves initially in air? Since the solubility of oxygen depends upon its partial pressure, and taking account of the nerves' metabolism, the amount of oxygen dissolved will be equivalent to about 0·004 c.c. per g. If dissolved oxygen were the cause of the "oxidative" heat in question, this would be reduced to less than one-fifth by using air. If the heat were due to a true oxygen store of some kind it would be nearly unchanged.

Calculated as above, the "oxidative" heat for the experiments of May 18, 20, 23 and 25 for the nerve initially kept in air are: 1945, 1470, 1909 and 2400 g. cm. per g. respectively; mean 1931 g. cm. per g. The amount of oxygen corresponding to 1931 g. cm. is 0·0091 c.c. per g. This is nearly twice as great as the amount which could initially have been dissolved in the water of the nerve.

What other source of heat could be suggested for the first stage of asphyxiation? One possible source is the breakdown of phosphagen to creatine and phosphate. According to Gerard and Tupikow [1930] labile phosphorus amounting to about 4 mg./100 g. is broken down in asphyxia. According to Hill's calculation [1932] this would liberate $1{\cdot}44 \times 10^{-3}$ cal. per g. of nerve, which at 10^{-3} cal. per g. per min. would last for 14 min. The quantity is so small that although it may have something to do with the first falling phase, it cannot explain it altogether.

It is difficult to say at present in what way the greater rate of asphyxiation, for the nerve kept previously in air, can be explained. It may be that the diffusion constant for the nerve is much smaller than is generally assumed, and more of the oxygen dissolved in the tissue could be used during asphyxiation before it diffused away; if so, however, owing to metabolism, the amount dissolved in the resting nerve would be

less; or it may be that the nerve produces less of the oxygen store if it is kept in air.

The question requires further investigation. At any rate, however, even if the diffusion constant for the nerve is so abnormally low that nearly all the oxygen dissolved in the tissue can be used by the nerve during asphyxiation, an amount of "oxidative" heat remains, which cannot be explained without the supposition that the nerve uses oxygen in other than molecular form.

Returning now to the second phase, this probably involves a breakdown of glycogen which is known to occur at an increasing rate, as shown by Gerard and Meyerhof [1927]. They found that the rate of lactic acid formation is slow during the first hour of oxygen want; about 3 hours from the beginning of asphyxia it occurs at a steady rate. It is interesting to calculate the amount of lactic acid to which the minimum heat rate of the asphyxiated nerve would correspond. The calculation suggests that *all* the heat production of this second phase is due to lactic acid formation. Taking (as above) 10^{-3} cal. per g. per min. as the minimum steady heat rate in asphyxia at 20° C., and assuming that the liberation and neutralization of 1 g. of lactic acid would lead to the production of 300 cal., the calculated lactic acid formation is $3{\cdot}3 \times 10^{-6}$ g. per g. of nerve per min., or 2×10^{-4} g. per g. per hour. Gerard and Meyerhof, working at 15° C., found over long periods of asphyxia a lactic acid formation of $1{\cdot}25 \times 10^{-4}$ g. per g. per hour. Applying a temperature coefficient of 2·5 to bring their results to 20° C. we get almost exactly 2×10^{-4} g. per g. per hour, corresponding to the number just calculated from the minimum heat in the present experiment.

When oxygen is readmitted to the asphyxiated nerve the excess heat is 2000–3000 g. cm. per g. This is several times as great as the heat (about 600 g. cm. per g.) liberated, as calculated above, by phosphagen breakdown. It may in part represent waste energy in the process of phosphagen resynthesis under the influence of oxidation. There is no indication at present of any other source of, or function for, this heat.

Summary.

1. The rate of resting heat production of frogs' nerve at 20° C. has been studied in oxygen and during oxygen lack.

2. In oxygen it is 176 g. cm. or $4{\cdot}14 \times 10^{-3}$ cal. per g. of nerve per min.

3. During asphyxiation the resting heat rate slowly diminishes, falling in 3–3½ hours to 20 or 25 p.c. of its initial value.

4. The mean value of this minimum heat rate during asphyxiation was 42·5 g. cm. or 10^{-3} cal. per g. of nerve per min.

5. When oxygen is readmitted extra heat is produced which is (after 3–4 hours anaerobiosis) between 15 and 20 p.c. of the heat missed during oxygen want.

6. The resting heat rate in oxygen corresponds to the oxygen consumption measured by other observers, and the minimum resting heat rate in nitrogen to the lactic acid formation observed by Gerard and Meyerhof. The smallness of the recovery heat after asphyxia agrees with Fenn's observation of the smallness of the recovery oxygen under the same conditions.

I am deeply indebted to Prof. A. V. Hill for his suggestion of the present work and for his helpful advice and criticism. My sincere thanks are due also to Mr J. L. Parkinson and Mr T. P. Feng for their willing assistance throughout the investigation.

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612.45

THE FUNCTION OF THE ADRENAL MEDULLA.

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We have reported in a previous note[2], that under certain conditions extracts of the adrenal medulla show an activity which cannot be explained by the adrenaline present. This activity was apparently due to a substance similar to adrenaline, but more potent than the latter. Till a chemical name can be substituted we propose to call this substance "Novadrenine."

It is not the object of the present paper to give an exhaustive analysis of the physiological activity of novadrenine. We only want to give fuller evidence of the existence of such a substance, and to call the attention of research workers to this field of investigation. The present authors feel incompetent to give a definite physiological analysis, and are greatly hampered in their work by the lack of material.

The existence of novadrenine is based on the observation, that under certain conditions the potency of adrenal extracts is 10–15 times higher than would correspond to their adrenaline content, estimated chemically by a colorimetric method. This high activity was only observed if the glands were excised soon after death and cooled carefully. In a great number of experiments the adrenaline content of the ox medulla was estimated colorimetrically and was found to lie between 1–2 mg. per g. of tissue, whether the extract showed a high physiological activity or not. In active extracts the physiological activity corresponded to an adrenaline content of 15–30 mg. of adrenaline per g. of medulla, a difference which could hardly be explained by an experimental error. If this latter value were correct, the gland would contain 10–15 p.c. of adrenaline by dry weight, which is impossible. It was also frequently observed that during chemical manipulations the excess activity of the extracts suddenly disappeared, the values obtained by chemical analysis and physiological estimation being then in close agreement.

[1] Holder of an American-Hungarian Exchange Fellowship, 1931–2, from the Institute of International Education, New York.

[2] *Nature*, April 9, 1932.

It was thus supposed that the potency of our extracts was partly due to the presence of a substance more potent than adrenaline itself. Whether this substance, novadrenine, is built up from adrenaline under certain conditions, or adrenaline is a cleavage product of the former, cannot be stated at present.

Owing to the lack of a specific chemical reaction for novadrenine, it cannot be stated in what proportion our active extracts contained adrenaline and novadrenine. Consequently only a minimum estimate of the relative activity can be given. If, for instance, our extract shows a potency ten times as great as that corresponding to the adrenaline content, we may conclude that novadrenine is at least ten times as active as adrenaline. This would be the case, if our extract contained only novadrenine, and if adrenaline and novadrenine gave identical colour reactions. If, however, only 1 p.c. of the adrenaline was present as novadrenine, the latter would be a thousand times as potent as adrenaline.

It was reported in our preliminary note, that, when given in small doses to the cat, novadrenine does not cause a fall of blood-pressure, and that in this respect its action differs qualitatively from that of adrenaline. This observation, however, could not be verified later, and there is no reaction known at present in which novadrenine behaves qualitatively in a different way from adrenaline.

Methods.

Material. All our experiments were made with ox suprarenal glands. The glands were excised as soon as possible after death and placed on ice and transported to the laboratory. Here they were chilled at − 20° C. The chilled glands were cut open, and medulla and cortex were separated. The medullary tissue was minced in a fine mincer with holes of 1 mm. diameter. The pulp was suspended in a ½ p.c. solution of trichloroacetic acid, 2 c.c. being used for every gram of the pulp. The suspension was rapidly heated to 80° C. and rapidly cooled again. The extract was filtered and the clear solution was kept in the refrigerator at − 20° C. Extracts were kept here for more than a week without apparent loss of activity.

Colorimetric estimation. The quantity of adrenaline present was estimated by the following method. The extract was neutralized with a small excess of sodium bicarbonate. Lugol solution was added, till the brownish red colour indicated an excess of iodine. The excess of iodine was removed by 0·1 *N* sodium thiosulphate, leaving a pink colour, which

was stable for a minute or so, and could be compared with the colour of a known solution of adrenaline.

This colour reaction was controlled with a number of generally used colour reactions, active extracts being compared with adrenaline solutions. We used the reaction with mercury chloride and the persulphate test of Ewins, both tests being based on the coloured oxidation product of adrenaline. We tested the ferric chloride reaction, in which the *o*-dihydroxy groups are estimated. The Folin-Dennis test was also used, which is based on the reducing power of adrenaline. All these tests gave identical results with our colour test. Only the Folin-Dennis test

Fig. 1.

gave higher values with extracts containing hexuronic acid, which acid readily reacts with the reagent. Adrenaline added to our extracts was readily indicated by our reagent.

Other methods of extraction, as by cold 5 p.c. trichloroacetic, hot 0·1 p.c. acetic, or methyl-alcohol showed no advantage. The first was as good as the hot $\frac{1}{2}$ p.c. trichloroacetic, the latter two were distinctly inferior.

Experiments.

If small doses of adrenaline are compared in the decapitated cat with active extracts containing colorimetrically the same amount of adrenaline, a much stronger pressor response will be given by the latter. If the dose of extract is gradually decreased, the dose of adrenaline being kept constant, it will be found that one-tenth to one-fifteenth of the dose of

Fig. 1.

Fig. 2.

extract will give a response equal to that with adrenaline. The same is the case in cats after decerebration or under ether anæsthesia, provided the animal does not give a depressor response, which makes the results incomparable.

Fig. 1 illustrates the point. In this experiment the action of 0·01 mg. of natural adrenaline is compared with that of a diluted extract, containing 0·001 mg. of adrenaline, as determined colorimetrically, in a cat under ether. (Time tracing, 3 sec.)

In Fig. 2 the action of 0·05 mg. of adrenaline is compared in a decerebrated cat with an extract, containing the same amount of adrenaline, by colorimetric estimation.

If the effects of big doses of adrenaline, producing a rise of blood-pressure to the maximal height, are compared with those of an extract giving an identical colour reaction, the response to the latter will show a much longer duration. This is illustrated in Fig. 3 in the rabbit (ether anæsthesia).

If active extracts are compared with adrenaline, for activity on an isolated loop of rabbit's intestine, the inhibitor action of the extract will be found much stronger. Equally strong inhibitor actions are produced by adrenaline and by the extract, when the latter contains 10–15 times less adrenaline as measured colorimetrically.

In the Trendelenburg frog preparation a similar difference is found in the vaso-constrictor activity.

Summary.

The fresh adrenal medulla contains under certain conditions a substance having a more potent pressor action on the blood-pressure, and a more potent inhibitor action on the isolated gut than adrenaline. The substance is called "novadrenine." The results are discussed and supported by figures.

We are indebted to Sir H. H. Dale for personal mention of some observations of his own, and for helpful criticism of our results.

This research was sponsored by the Ella Sachs Plotz Foundation.

NOTE ON THE PREPARATION OF CRYSTALLINE ADRENALINE.

By the following method this can be done without the use of alkali. It is hoped that the separation of crystalline novadrenine, in the hands of workers in command of suitable material, may thus become possible.

The trichloroacetic extract (300 c.c.) is neutralized with NaOH and concentrated *in vacuo* at a low temperature to a small volume (3–5 c.c.). Gradually about 50 c.c. of methyl-alcohol are added, the inactive precipitate separated, and the fluid concentrated again *in vacuo* to an oily liquid. To this, 5 c.c. of methyl-alcohol and then 15 c.c. of acetone are added. The oily precipitate is separated on the centrifuge. To this precipitate again 5 c.c. of methyl-alcohol are added. After centrifuging off the inactive precipitate, 15 c.c. of acetone are added to the fluid. Again the precipitate is separated and discarded. To the two combined acetone-alcohol solutions a solution of ferrous chloride in aqueous acetone is added. This ferrous reagent is prepared by adding 9 parts of acetone to a saturated watery solution of the salt. After the precipitated excess of the salt has settled, the supernatant clear liquid is used.

On addition of the ferrous reagent a deep purple-blue precipitate separates consisting of the ferrous complex of adrenaline. This precipitate forms only at neutral reaction. On addition of the ferrous reagent the solution turns acid and must be neutralized with an acetone solution of ammonia (1 part of strong watery solution of ammonia and 4 parts of acetone). The ferrous reagent and ammonia are alternately added till, at neutral reaction, no more coloured precipitate is formed in the presence of iron. The precipitate leaves a slightly coloured blue liquid behind. The precipitate is separated on a Büchner funnel, suspended in little water (15 c.c.), and a vigorous current of H_2S is passed through. The fluid is quickly filtered on a Büchner. Very soon the crystallization of adrenaline begins, which is completed by cooling in an atmosphere of nitrogen.

The mother liquor contains only about 0·2 mg. of adrenaline per c.c. The yield is nearly 2 mg. of adrenaline per gram of medulla, which is not far from the total adrenaline content of the gland. The method can be applied with the same result to extracts of the whole gland.

If the preparation of novadrenine is attempted, possibly low temperatures should be applied for the concentration of the extract. Novadrenine is also carried down by the ferrous reagent, and can be liberated by H_2S without losing its activity.

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THE SUPERNORMAL PHASE IN MUSCULAR CONTRACTION.

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The isometric response of a muscle to a single shock can be measured, not only by the maximum tension developed, but by the area of the tension-time curve. The importance of tension-time is that it is the basis

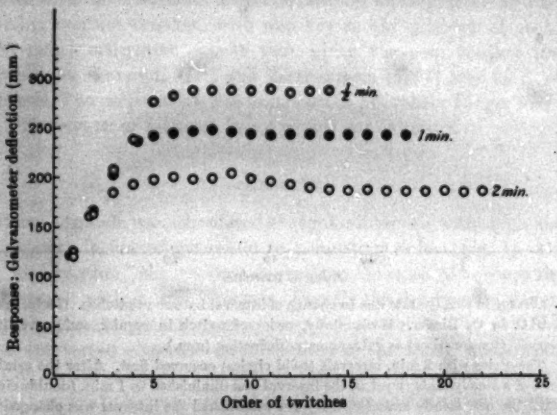

Fig. 1. Attainment of steady state during regular series of twitches. Gastrocnemius at 0° C. in O_2 Ringer. Horizontally, order of twitch in regular series; vertically, response (tension-time) as galvanometer deflection (mm.).

The three series were made in order: 2 min. intervals, 1 min. intervals, ½ min. intervals. After the first two series a long rest was given.

of the maintained contraction: the "economy" of a tetanus depends upon the "area" of a single twitch. The method described below allows the integration necessary for determining the tension-time to be performed instrumentally and the results to be read as the ballistic deflection of a galvanometer.

Recorded as tension-time the response of a muscle to a single shock shows a striking supernormal phase [Hartree and Hill, 1921] in the sense that at a suitable interval the response to a second shock is greater than that to the first one. Other aspects of this supernormal state have been referred to recently by Hill [1931 *a*, p. 296] and by Rushton [1932, p. 244]. In the present research its characteristics have been further investigated.

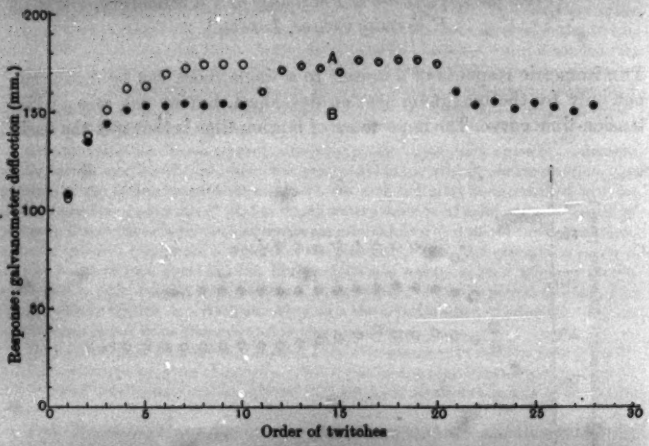

Fig. 2. Change in steady state due to change of interval between twitches. Gastrocnemius at 0° C. in O_2 Ringer. Horizontally, order of twitch in regular series; vertically, response (tension-time) as galvanometer deflection (mm.).

The series with 2 min. intervals (solid circles) occurred first. After the establishment of a steady state (level *B*) the interval was diminished to 1 min. (double circles). When the new steady state (level *A*) was established the interval was changed back to 2 min. and a third steady state was found at the same level *B* as before (solid circles). After about one hour's rest the muscle was stimulated again in a regular series at 1 min. intervals (hollow circles); the steady state was now reached at level *A*.

If a regular series of shocks be given to a muscle at not too high a rate the tension-time response (hereafter referred to as the response) increases until it reaches (apart from the onset of fatigue) a stationary level (see Fig. 1). Increasing the interval then causes a fall, decreasing the interval a rise in the level (see Fig. 2). If at any moment after one of the shocks of such a series an extra shock be interpolated, the response is greater or less according to the relations described below.

Method.

A differential cuprous oxide photoelectric cell was employed, as suggested by Hill [1931 *b*]. It was joined to a slow sensitive moving coil galvanometer employed ballistically. The gastrocnemius of a small Dutch frog (*R. esc.*) was connected by a wire to an isometric spring myograph, from the mirror of which a strong beam of light was reflected on to the middle of the cell. When the muscle contracted, the beam of light moved and generated a current in the cell. The amount of current so generated and recorded by the galvanometer was proportional to the tension-time.

The muscle was stimulated by single super-maximal induction shocks through its nerve. Two Harvard coils placed at right angles on the table were adjusted to give identical shocks, their secondaries being arranged in series with the electrodes. The time interval was regulated by a Lucas revolving contact breaker, with one key in the primary of each coil. When many successive shocks were given the cam contact breaker described by Gerard, Hill and Zotterman [1927] was used with a single coil. The preparation was immersed in phosphate Ringer's solution (*p*H 7·2), oxygen or nitrogen being passed continuously.

Results.

When a muscle was stimulated at regular intervals with single shocks, the response increased gradually to a constant value (Fig. 1). During this "steady state" the response depends on the interval between shocks, being greater with shorter intervals. Usually the steady state was stable, except in the absence of oxygen, when fatigue set in. The supernormal phase was tested after the establishment of the steady state by interpolating an extra shock. The muscle then had to contract twice in one interval, so that the next regular shock gave a slightly greater response. Continuing, however, with the regular series the steady condition was re-established.

Following this routine, the supernormal effect at the second of two stimuli was determined with various intervals. Two different stages are found: (*a*) the relatively refractory phase, in which the second response is less than the first; (*b*) the supernormal phase, lasting up to the next regular shock, in which the second response is the greater. A third, but artificial, subnormal phase is then found in which, owing to the necessary omission of the next regular shock if an interval greater than that of the series is to be employed, the response is less than in the series.

TABLE I. Gastrocnemius at 0° C. in O_2 Ringer: regular series at 1 min. intervals. The supernormal value was obtained by subtracting double the regular response from the summated response (or when possible the regular response from that to the interpolated shock). With intervals more than 2 sec. the galvanometer was short-circuited during the first response; then the short-circuit was opened, so as to obtain the second response only.

Interval between shocks (sec.):											
0·1	0·75	1·25	1·75	7	20	30	45	60	75	90	120
Supernormal effect (p.c.):											
−63	+36	+34	+34	+35	+15	+13	+4	0	−2	−7	−10

When, during a regular series of shocks, a single one is replaced by a group in rapid succession, the extra activity disturbs the steady state and the next response in the regular series is enhanced.

TABLE II. Gastrocnemius at 0° C. in O_2 Ringer: regular series at 1 min. intervals. Excess of response at next regular shock as the result of extra activity immediately following a shock of the regular series. (Extra activity represented by number of extra shocks at 0·5 sec. intervals.)

Extra shocks	1	2	3	6	9	14
Excess response (p.c.)	5	8	11	15	18	20

The supernormal phase following a single shock can clearly be regarded as the first step in the establishment of a new steady state. This is true even of as short an interval as 0·5 sec. at 9° C. In such a case each shock is applied during and not after the previous contraction, and the total effect of a group of shocks must be read as a whole. With the longer groups the galvanometer had to be employed beyond the range of its ballistic proportionality, and a correction was employed by means of an experimental curve relating deflection to duration of illumination

TABLE III. Gastrocnemius at 9·4° C. in O_2 Ringer: regular series at 1 min. intervals.

Sequence of observations		No. of extra shocks 0·5 sec. apart	Mean supernormal effect of extra shocks p.c.	Average p.c.
1			35	
2			35	34
	12	1	33	
3			65	
	11	5	86	76
4			104	
	10	10	129	117
5			136	
	9	20	135	136
6			156	
	8	30	140	148
7		40	133	133

of the photoelectric cell by an unsymmetrical light spot. See Fig. 3 and Table III.

At higher temperatures the phenomena of the supernormal phase are not so evident, and the time intervals at which they must be sought are much less. At 20° C., for example, an interval of 5 sec. should correspond approximately to one of 1 min. at 0° C. In the paper by Hill [1931 *a*, Fig. 9, p. 296] there are obvious supernormal responses to the later shocks of a 0·9 sec. and a 0·6 sec. series at 20·2° C. Since the

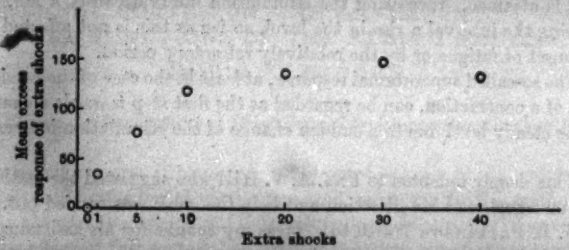

Fig. 3. Constructed from Table III. Horizontally, number of extra shocks at 0·5 sec. intervals given immediately after one of a regular series of shocks at 1 min. intervals; vertically, mean supernormal effect, *i.e.* mean response of extra shocks in excess of response in regular series expressed as a percentage of the latter. Gastrocnemius at 9·4° C. in O_2 Ringer.

intervals required are longer and the phenomena are more evident at the lower temperature, attention in the present research has been directed chiefly to these.

Discussion.

It has been shown that the tension-time response is greater, after a short interval during which it is less, the less complete the return of the muscle is to its resting or steady condition at the moment of response. There are two factors in this increase of the area of the tension-time curve, namely the usual staircase effect and the "slowing" effect recently referred to by Hill [1931 *a*, Fig. 9]. The latter factor seems to be much the more important.

The supernormal phase in the thermal response and that in the mechanical response are independent of each other [see Hartree and Hill, 1921, Fig. 6]. There are various other forms of response in which a supernormal phase has been reported, *e.g.* in the electric response

[Samojloff, 1908; Adrian and Lucas, 1912], in the height of isotonic contraction under certain conditions [Bremer and Homès, 1932] and in the return of excitability [Adrian and Lucas, 1912; Cooper, 1924]. Whether there is any connection between the various supernormal phenomena is not certain.

SUMMARY.

If a regular series of single shocks be given to a gastrocnemius muscle through its nerve its tension-time response increases until a stationary level is attained. Increasing the stimulation interval causes a fall, decreasing the interval a rise in the level, so far as this is not affected by the onset of fatigue or by the relatively refractory period.

The so-called supernormal response, at least in the case of the tension-time of a contraction, can be regarded as the first step towards a change in the steady level due to a sudden change of the stimulation interval.

I am deeply indebted to Prof. A. V. Hill who suggested the method, and under whose kind direction and help the work was carried out. To Mr J. L. Parkinson I wish to express my thanks for his assistance in the technique.

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THE EFFECT OF INJECTIONS OF EXTRACTS OF ADRENAL CORTEX ON THE DEVELOPMENT AND SEX FUNCTIONS OF THE ALBINO MOUSE.

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In a previous paper [Cleghorn, 1932] proof is given of the activity of extracts of beef adrenal cortex prepared according to the method of Swingle and Pfiffner [1931]. It was indicated there that observation of the effect of this material on the sex functions supplied an excellent means for the experimental test of the averred relationship between them and the adrenal cortex. This has already been tried but with conflicting results. Connor [1931] found that rats injected with Swingle and Pfiffner's extract matured no faster than controls, as judged by the time of opening of the vagina, vaginal smears in the female and the behaviour of males to females in œstrus. Nor did he find the œstrous cycle of adult rats to be interrupted by injections of the extract. Previously [1930] he had reported that the injection of hens with a crude watery extract of beef adrenal cortex caused cessation of laying, atrophy and degeneration of the ovaries and delay in the development of young chickens. This extract, he claims in his later publication, interrupts the œstrous cycle of rats.

Contrary to Connor's [1931] report are the findings of Corey and Britton [1931 *a*, *b*]. These workers claim to have produced precocious maturation of the sex glands of young albino rats by injections of Swingle and Pfiffner's extract. Changes were not so marked in the testes as in the ovaries. The weight increases of the test animals were from 10 to 18 p.c. greater than those of the controls.

The work herein described constitutes a further attempt at the experimental elucidation of the averred relationship between the adrenal cortex and the gonads, using extracts of adrenal cortex.

Material.

The albino mouse was selected as the test object on account of its convenient size and easily observed sex phenomena. The chief extract used for injecting the mice was prepared according to the method of Swingle and Pfiffner, to which reference has already been made. A crude watery extract was used in a few experiments and these are detailed in part (*b*).

The mice forming the initial stock were secured from a reputable London dealer. They were kept in a warm room, temperature 70–80° F., and fed regularly on a diet of bread soaked in milk, crushed oats and millet seed, with a constant supply of water. Under these circumstances regular œstrous cycles were observed and no difficulty experienced in breeding the animals. Our experience accords with that of Wyman [1928] and others, namely that low temperature and inadequate diet interfere with the reproductive functions of the rodent.

(*a*) The effect of injections of extracts of adrenal cortex on the growth and maturation of mice.

The criteria selected for judging the effect of the injected extracts were:

(1) Growth (body weight and testis weight).

(2) Maturation. Age of opening of the vagina. This is held to be an accurate index of physiological maturity since, according to Engle and Rosasco [1927], œstrus, and hence ovulation, usually occurs within 24 hours after the vagina opens.

Of the litters of ten mice, totalling 55 young, 39 were used—18 as test, and 21 as control animals.

Two litters born on the same day constituted an experimental unit of test and control groups. There were four such units. The size of the litters was equalized at birth by discarding from the larger, and in three of the units half the young were transferred from the prospective test group to the control group and *vice versa*, in order to control genetic factors which might influence growth and sex development. The litters in the fourth unit were not interchanged, but the results are so similar to the others in detail as to make it probable that consideration of the genetic factor is unnecessary. Two additional litters were observed as extra controls.

All injections were administered subcutaneously and no untoward

reaction was seen to occur. Of the six mice that died two were killed by the needle and four from unknown causes. Injections of beef cortical extract 1/20 c.c. twice daily, *i.e.* the equivalent of the cortex of one beef adrenal (6 g.), were administered throughout the lactation period of 3 weeks to the mother mouse of the test litter. The mother of the control group was injected with extract inactivated by boiling, or with saline in equal amounts. The young mice were weighed on weaning and twice weekly thereafter. Injections were begun on the 21st day, the test mice receiving 1/20 c.c. active extract twice daily for 2 weeks, then 1/10 c.c. twice daily for 2 weeks. The controls were injected with inactivated extract or saline in similar amounts.

Results.

Only the initial and final body weights need be given along with the combined weight of the testes taken at the end of the experiment.

TABLE I.

	Males						Females			
	Initial wt. (g.)		Final wt. (g.)		Combined wt. Testes (g.)		Initial wt. (g.)		Final wt. (g.)	
Exp. No.	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
I	—	6·0	—	13·7	—	0·12	7·7	7·4	18·4	16·3
	—	7·3	—	19·0	—	0·15	7·8	—	17·6	—
	—	5·8	—	15·4	—	0·12	7·4	—	15·7	—
							7·1	—	14·5	—
							7·2	—	16·2	—
							7·0	—	15·8	—
II	8·1	7·2	17·3	19·0	0·16	0·185	8·0	8·8	20·1	16·0
	7·4	7·9	17·0	16·8	0·15	0·185	7·3	9·3	17·7	16·0
	7·0	—	15·9	—	0·13					
III	4·6	3·8	13·6	12·9	0·10	0·10	5·5	5·5	11·1	13·9
							5·6	3·7	13·8	12·2
							5·5	4·9	Died	13·4
IV	8·2	9·2	14·5	18·2	0·125	0·185	8·0	7·7	16·0	14·4
	8·0	7·7	16·7	18·3	0·125	0·165	—	8·2	—	15·3
Extra Control Group I		7·1	—	14·6	—	0·12				
		6·6	—	13·4	—	0·12				
		7·7	—	12·9	—	0·12				
Average	7·2	6·9	15·8	15·8	0·131	0·143	7·0	6·9	16·1	14·7

Initial weights on 21st day. Final weights in Exp. I on 51st day, others on 40th.
Extra Control Group II is not included here since final weight was taken on 40th day.

Consideration of Table I shows the average weight of the testes of the test animals to be 8·3 p.c. less than that of the controls, and the final weight of the test females 8·7 p.c. greater than the controls.

Table II shows that there was no substantial difference between the test and control groups in either average weight or age at the time of opening of the vagina. The average of 31·5 days for the occurrence of

TABLE II.

	Weights at vaginal opening (g.)		Age at opening (days)	
Exp. No.	Test	Control	Test	Control
I	12·5	13·4	31	31
	12·8	—	31	—
	11·8	—	31	—
	11·8	—	38	—
	11·7	—	38	—
	10·5	—	31	—
II	12·3	10·8	29	30
	12·2	12·4	29	32
III	9·8	10·9	35	37
	9·8	7·5	35	33
	9·6	8·6	33	33
IV	12·7	10·4	29	29
	—	12·6	—	32
Extra Control Group II	—	13·5	—	30
	—	14·5	—	28
Average	11·5	11·4	32·5	31·5

this phenomenon in the controls appears early compared to the range of 6–7 weeks given by Parkes [1929], but is in close agreement with that of Engle and Rosasco [1927], who found that the first appearance of œstrus in 100 mice occurred from the 28th to the 49th day, with a median at the 35th day. This discrepancy may be explained by genetic factors [Mirskaia and Crew, 1930]. It may be mentioned here that the term "sexual maturity" does not necessarily imply the occurrence of first œstrus and ovulation. Parkes [1925] has used it "to mean the capacity of the sex organs to function."

(*b*) The effect of injections of extracts of adrenal cortex on the œstrous cycle.

The œstrous cycle was observed by the vaginal smear technique in a number of adult female mice for a period of weeks. The periods of cornification, or œstrus, and the diœstrous interval varied in different animals, but the usual length of the cycle was 5–7 days. All injections were made subcutaneously.

(1) *Effect of Swingle and Pfiffner's extract.*

Exp. i. Six mice with regular cycles were injected daily for a week with 1/10 c.c. of extract inactivated by boiling, and then with a similar amount of active extract for 2 weeks. In the chart below the œstrous cycles are recorded just before and during the experiment:

Mouse No.	
5	00000 – – 0I00 – – 000 | A0 – – 00 – – – – – – – – 0 | 00 – – – – – 00 – – 0000
6	0000 – I – – 0000 – – | A – – – – – – 00 – – 0000 | – – – 0000
30	0000 – – 00I0 – – – – – – | A0 – – – – – 000 – – – – – | – – – – 000
1	0000 – – – I – 000 – – – | A000 – – – – – – – – 0000 | – – 00
11	00 – 0I00 – – – 00 | A0 – – – 00 – – – – – 000 – | – – – – – – 000
17	000000 – – 00I0 – – 000 – | A – 0000 – – 00000 – – | 0000

0 means œstrus; – dicestrus; I injections of inactivated extract begun; at A daily injection of active extract begun. The vertical line indicates cessation of injections.

The obvious objection to this experiment is that an interval was not allowed between the control and test injections, so that it is difficult to say whether the subsequent disturbances which sometimes occurred in the cycles were due to the specific influence of the active extract or merely to the injections *per se*. The latter interpretation appears probable from the occurrence of the prolongation of the dicestrous interval in No. 30 during the injections of inactivated extract and the absence of any disturbance of the cycle of No. 17 with either type of injection.

Exp. ii. This was performed with five mice, two of which had been used in Exp. i whose cycles were of proved regularity. The remaining three mice had somewhat less regular cycles. They all got 1/5 c.c. of active extract twice daily.

Mouse No.	
1	00 – – 000A – – – 000 – – – – – 0000 | – – – – – – – – – –
2	0000 – – A000000 – – – 0000 | 00 – – – 00000
32	00 – – 0000 – A – 00000 – – | 000
33	0 – – – – – – – – 00A000000 – – | – – 00
34	0 – – – – – – 0A00 – – – 00 – | – – – – – – – – – (scabs)

The large dose of extract injected did not summarily terminate œstrus in any of the mice, although prolongation of dicestrus is noticeable in No. 1. This mouse looked dirty and unkempt and had but two periods of œstrus in the subsequent 6 weeks. No. 34 developed scabs, sufficient explanation for the prolonged dicestrus, and had to be killed.

It is apparent then that injections of this type of extract of beef adrenal cortex do not immediately terminate œstrus. The frequent prolongation of the dicestrous interval seen during or following such in-

jections cannot be held to be specifically the effect of the extract *per se*, since control experiments in which inactivated extract was used gave similar prolongation, and we have also observed this with saline injections. Connor [1931], too, has recorded a similar occurrence in rats with adrenaline injections.

Connor also reports an experiment in which 13 female rats were injected daily with 2 c.c. of extract made by macerating fresh beef cortex with an equal amount of Ringer's solution and filtering through a Seitz, with the result that œstrus was suppressed in seven, and appeared irregularly in the other five. These results were the reason for the following experiment:

(2) *The effect of watery extract of beef adrenal cortex.*

Exp. For each day's injection of extract fresh glands were secured except on two occasions when glands were not available, so that extract made on one day had to be used on the second also. Dissected cortex was minced and mixed with an equal amount of Ringer's solution and left in the refrigerator for an hour. Mice Nos. 12, 17, 32, 33 and 35 were injected twice daily for 3 days with 0·3 c.c. of the fluid filtered from the above gland mixture through a coarse filter paper in a Büchner funnel. Subsequently they, and the other mice from the beginning, received 0·4 c.c. twice daily of extract obtained by centrifuging the fluid strained through muslin from the gland-Ringer mixture and filtering through a Seitz.

Since an adult mouse has a weight of about one-seventh that of an adult rat, the amount of the dose is relatively greater than that used by Connor.

The œstrous cycles of the mice before and after injections are recorded below:

Mouse No.	
12	000 – 0T0000 – 00 – – dead
17	0 – 00T000 – 00000 – – – – 0 | 0 – – –
32	0 – – 0T00 – – – – 0000 | 00000
33	0 – – – – T000 – – – – – | 00000
35	0 – T000 – – 00 – – 0 | – – – –
42	– 0000 – – T00000 | – – – –
52	00 – – – T0000 – – – – | – – – –

T means injections started. Vertical line, stopped.

It is evident that in no case did œstrus cease immediately injections were begun, as noted by Connor in his similar experiment with rats. Œstrus also recurred in five of the mice during the period of injections.

In two cases œstrus did not recur during the period of injections or in the days of observation subsequently. There is a tendency to prolongation of the dioestrous interval.

Discussion.

These results indicate that injections of Swingle and Pfiffner's extract of adrenal cortex do not affect growth and maturation in mice, and they are substantially in agreement with those of Connor in which rats were used. The occurrence of considerable individual variation makes questionable the significance of the observations that the average weight of the testes in the test group was 8·3 p.c. less than in the controls, and that there was a preponderance of 8·7 p.c. in body weight of the test females. On the other hand the excess weight of 10–18 p.c., shown by the test groups to controls in Corey and Britton's experiments, may be significant, for these workers also showed that the ovaries of injected animals were stimulated to mature more rapidly than normal. However, the fact that opening of the vagina in our test animals did not occur at an earlier date than in the controls is held to be sound evidence that maturation cannot have been accelerated.

In seeking an explanation for the apparent discrepancy in these results, the question of dose first arises. Corey and Britton record early maturation and 10 p.c. weight excess in litters 11 and 13 in which the dose was 1 c.c. on alternate days. The average weight of these rats was about 50 g. The average weight of the mice at the same age (5 weeks) was about 13 g., the dose 1/10 c.c. daily, though they subsequently received twice this amount. These are closely comparable doses, presuming that the extracts were of equal strength, and as shown elsewhere [Cleghorn, 1932] the extract used in these experiments was effective in combating adrenal insufficiency in cats; as little as 7·5 c.c. restoring a prostrate cat to activity. At the same time it must be noted that the extracts used in the foregoing experiments contained a substance, probably histamine, having a deleterious effect on young rats and mice when injected intraperitoneally, and that this may have masked any growth or maturation effect that might otherwise have been evident.

Concerning the effect of cortical extracts on the œstrous cycle, the results with the Swingle and Pfiffner extract reported here are in accord with Connor's findings. Such disturbances of the œstrous cycle as are seen in some of the mice may be fairly considered non-specific for reasons stated. The marked interference with the cycle of rats

injected with watery extract reported by Connor is not confirmed by similar experiments with mice. We venture to suggest that the interference may be due to non-specific causes and not evidence of a "sex-affecting portion" of the adrenal cortex.

Summary.

1. The final average weight of young female mice, injected daily for a period of 4 weeks with an extract of adrenal cortex made according to the method of Swingle and Pfiffner, is 8·7 p.c. greater than the controls. There was no observable difference in the final average weight of test and control males. The final average weight of the testes of the test males is 8·3 p.c. less than that of the controls. These weight differences are not held to be significant.

2. The injection of this type of extract has no appreciable influence on the age of maturation of young female mice as judged by the opening of the vagina, nor has it any specific effect on the sex function of the adult female mouse as judged by its effect on the œstrous cycle.

3. The injection of a watery extract of beef adrenal cortex does not cause the cessation of œstrus, and the frequent prolongation of the diœstrous interval that follows such injection can only be attributed to non-specific influences.

I wish to thank Prof. J. J. R. Macleod, F.R.S., for his helpful criticism, and Dr A. S. Parkes for advice on several points.

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THE REFRACTORY PERIOD OF MAMMALIAN CARDIAC MUSCLE, WITH ESPECIAL REFERENCE TO PURKINJE TISSUE.

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The observations of Mines [1913] on the frog's heart suggest that the refractory period of the ventricular muscle shortens as the rate of beating is raised. Lewis, Drury and Bulger [1921] demonstrated this relation in the auricular muscle of the dog, while recently Tso [1930] has published some observations on the frog's ventricle which confirm the findings of Mines [1913].

That the refractory period decreases as the rate of beating is raised, appears to be a property of cardiac muscle.

In all these observations the stimulating shocks, which test the refractory period, have been sent in at one point, and the response of the muscle has been recorded either from paired electrodes placed a short distance away on the muscle [Lewis, Drury and Bulger, 1921] or from electrodes placed upon the base and apex of the heart [Mines, 1913; Tso, 1930]. A conduction factor is therefore introduced, and the refractory period measurement is not necessarily of the earliest moment at which the muscle beneath the stimulating electrodes will respond, but of that at which the response will travel to a distance through the muscle. From the point of view of the heart beat, this measurement is of interest, but the conduction factor must not be overlooked when the actual properties of the muscle cell are under consideration. It is probable that, under physiological conditions, identical measurements will be obtained with the above method and with those in which the response is recorded at the actual point of stimulation [Andrus and Carter, 1930]. When the heart is poisoned by drugs the two measurements will differ, for it has been shown by Drury and Love [1926] that, in the veratrine poisoned frog's heart, the muscle beneath the

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[2] Working on behalf of the Medical Research Council.

stimulators responds at a moment considerably earlier than that at which its response can travel even a short distance away.

To distinguish between these two refractory period measurements Lewis and Drury [1926] suggested the terms "effective refractory period" for those measurements in which the response must be conducted to a distance, reserving the term "absolute refractory period" for measurements in which the conduction factor is eliminated.

In this paper we are primarily concerned with observations upon the refractory period[1] of the perfused rabbit's ventricle. In earlier experiments we were unable to find the accepted relationship between refractory period and the rate of beating. We thought that this might be due to the fact that the nutritive and mechanical conditions are very different in the perfused organ, but this conclusion was not supported by further experiments. These and associated experiments have led us to conclude that, in such a preparation, the refractory period measurements obtained are not those of ventricular muscle but of a mixed tissue containing muscle and Purkinje fibres.

The perfused rabbit's ventricle.

The rabbits were killed by a blow on the head, and the hearts quickly removed from the thorax. They were perfused in the usual manner through a cannula inserted into the aorta, with a Ringer's fluid of the following composition: NaCl, 0·92 p.c.; KCl, 0·04 p.c.; $CaCl_2$, 0·024 p.c.; $NaHCO_3$, 0·015 p.c. This fluid was brought to a *p*H of 7·2–7·4, and was continually oxygenated by bubbling oxygen through it.

Although such evidence as exists strongly suggests that the vagus fibres do not influence the ventricular muscle [Drury, 1923; Rothberger and Scherf, 1930; Dale, 1930], it has been shown that the rhythmic shocks stimulate the vagal fibres in the auricular muscle of the dog and definitely influence the refractory period [Lewis, Drury and Bulger, 1921]. Atropine sulphate was added as a measure of precaution, in doses of 0·2–1 c.c.of a 1 p.c. solution to each litre of perfusion fluid.

The refractory period was measured in the following manner. Fish-hook electrodes were fixed into the epicardium of either the right or left ventricular muscle and paired non-polarizable electrodes connected to a galvanometer string applied a short distance away. The heart was

[1] All our measurements are of the "effective refractory period" except those in which the contrary is stated.

driven rhythmically from the fish-hook electrodes and an extra shock about ten times threshold value passed through the same electrodes, using the apparatus already described [Drury, 1925]. With a double string carrier in the galvanometer the shocks were recorded with one string, and the response of the ventricle simultaneously with the other[1]. To maintain a zero pressure in the ventricles, openings were freely made into the chambers. The sino-auricular node was cooled by placing a small metal tube upon it, through which ice-cold water flowed, or alternatively the A.-V. bundle was cut. This gave a slow natural rhythm and allowed the period to be tested at low rates of beating. As the preparation is slowly deteriorating, and the temperature of the heart susceptible to change with varying coronary flow, observations over a range of rates must be made as quickly as possible. It is only justifiable to compare values obtained in such a series and not those obtained at different stages of the same experiment.

The results typical of such experiments are shown in Table I. From these measurements it is clear that the absolute refractory period of both right and left ventricular muscle remains relatively constant over a considerable range of rates. In some experiments (0.8.12 *a* and *b*), a slight decrease in the period occurs as the rate of beating is raised, but it is not so great as would be expected for the heart *in situ* over the same range of rates.

The perfused heart, even with a well-oxygenated saline, must be considered relatively anoxæmic. To determine whether this was a factor in the results, blood Ringer was used as a perfusate. The animal was anæsthetized and bled from the carotid artery prior to removal of the heart. This blood was defibrinated and added to the perfusate, in amounts varying from 20 to 30 c.c. to 1 litre of Ringer's solution. The measurements obtained in these experiments did not differ materially from those obtained from hearts perfused with Ringer's solution. In two experiments the heart was perfused with whole defibrinated blood, for apart from the question of oxygen supply the saline perfusates differ from blood not only in their chemical composition but also in their viscosity, and it was thought that this might exert an influence. It was found possible, by returning the blood to the perfusion bottle immediately it left the heart, to work with 200 c.c. of defibrinated blood. Of this 150 c.c.

[1] In later experiments, the second string was dispensed with, the primary current of the stimulators being passed through a low-resistance loud speaker unit. The movements of the reed corresponding to the passage of the current were photographed simultaneously with the record of the galvanometer string.

TABLE I.

Exp. 0.8.1. Right ventricle.

Rate per min.	72	136	67	94	120	200
Response	0·135	0·133	0·139	0·134	0·136	0·137
No response	0·103	0·122	0·109	0·125	0·126	0·118
R.P.* in sec.	0·119	0·127	0·124	0·129	0·131	0·126

Exp. 0.8.5. Right ventricle.

Rate per min.	79	84	84	167	214
Response	0·21	0·22	0·20	0·21	0·21
No response	0·16	0·18	0·19	0·20	0·19
R.P. in sec.	0·185	0·200	0·195	0·205	0·200

Exp. 0.8.12. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)		(*b*)	
Rate per min.	107	214	86	197
Response	0·226	0·196	0·250	0·212
No response	0·211	0·185	0·230	0·182
R.P. in sec.	0·218	0·190	0·240	0·197

Exp. 0.8.12 *a*. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)				(*b*)		
Rate per min.	60	80	115	120	58	77	125
Response	0·192	0·198	0·185	0·188	0·222	0·209	0·192
No response	0·177	0·172	0·172	0·168	0·204	0·185	0·175
R.P. in sec.	0·185	0·185	0·178	0·178	0·213	0·197	0·183

Exp. 0.8.14. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)			(*a*)		(*b*)		(*b*)	
Rate per min.	83	166	250	107	214	100	200	100	200
Response	0·120	0·128	0·115	0·135	0·132	0·178	0·174	0·182	0·174
No response	0·107	0·124	0·104	0·119	0·121	0·176	0·157	0·171	0·160
R.P. in sec.	0·113	0·126	0·109	0·127	0·126	0·177	0·164	0·176	0·167

* This figure is the mean between the longest "no response" and the shortest "response." As each successive testing shock is usually delayed by about 0·01–0·020 sec., the error may amount on occasions to, but not more than, 0·020 sec. In subsequent tables the mean figure is alone tabulated.

were obtained from the ear veins of five rabbits, and another 50 c.c. from the rabbit supplying the heart for perfusion, by bleeding it from the carotid artery, introducing saline intravenously, and rebleeding. The different samples were defibrinated and mixed. The heart was removed, washed through with saline, and then connected with the perfusion apparatus. The perfusion circuit was continuously maintained by catching the blood immediately it had left the heart, and carrying it up a glass tube into the perfusion bottle, by means of a continuous stream of oxygen bubbles. The perfusion pressure obtained by this method was about 40 mm. of mercury, and the hearts were beating well at the end of 2 hours. The measurements of the refractory period which were made at different intervals after perfusion commenced showed that such a

preparation did not differ from those perfused with saline (Table II). It is evident therefore that neither anoxæmia, the lessened viscosity, nor the chemical constitution of the saline perfusates can be considered the cause of the constant refractory period.

TABLE II.

Exp. 1.12.9. Rabbit's heart. Blood perfusion.

Rate per min.	290	310	380	450	300	360	280	178	216	300
R.P. in sec.	0·102	0·105	0·103	0·107	0·101	0·103	0·126	0·132	0·141	0·131

The whole perfused heart presents practical difficulties for two reasons. Firstly, very low rates of beating cannot be tested, and secondly, at high rates ventricular fibrillation is often set up by the testing shocks which fall immediately after the refractory period has terminated.

A few observations therefore have been made upon muscle strips. Such preparations possess the advantages that they are quiescent and that ventricular fibrillation does not develop readily. The strips, prepared in the manner already described [Dale, 1930], were immersed in a saline bath, kept at a temperature of 37° C. They were placed resting on the bottom of the bath in a horizontal position, and two parts of the epicardium—namely that stimulated and that to which the recording electrodes were applied, were kept just above the level of the saline. The results, as is seen from the measurements given in Table III, show no

TABLE III.

Exp. 1.1.23 *b*. Left ventricular strip.

Rate per min.:	27	21	43	75	35	50	100	60	120	75	15	30	150	75
R.P. in sec.:	0·19	0·20	0·19	0·17	0·16	0·17	0·17	0·17	0·17	0·18	0·17	0·18	0·16	0·17

Exp. 1.2.19 *b*. Left ventricular strip.

Rate per min.:	50	150	300	62	187	374
R.P. in sec.:	0·08	0·08	0·09	0·08	0·08	0·08

appreciable difference from those obtained from the whole heart, the refractory period remaining constant over a considerable range of different rates, though there is a tendency for the duration to be slightly less at high rates than at low rates of beating.

THE INTACT RABBIT.

As the above results did not agree with those found in the frog's ventricle and the dog's auricle it seemed desirable to measure the period in the intact rabbit.

The rabbits were anæsthetized with urethane injected subcutaneously, followed, if necessary, by ether. Under artificial respiration, the chest was opened and the pericardium cut. The free edges were sutured to the chest wall making a sling in which the heart rested. The fish-hook stimulators were fixed in the epicardium of the right or left ventricle and the recording electrodes placed a short distance away on the same chamber. The natural heart rate was slowed by cooling the sinus or by burning it with a cautery. As the latter procedure is liable to perforate the auricle, the cooling method is preferable. Atropine sulphate (½ c.c. of a 1 p.c. solution) was injected intravenously. The results in such a preparation usually showed that the length of the period changed as the rate was raised. In certain animals, however, measurements were obtained which indicated that the period was relatively constant, but these results were unusual. In Table IV the measurements typical of these

TABLE IV.

Exp. 1.2.6. Intact rabbit. Atropinized.

Rate per min.:	88	167	176	190	200	200	214	230	250	273	273	375
R.P. in sec.:	0·173	0·122	0·133	0·132	0·111	0·110	0·118	0·107	0·107	0·091	0·096	0·086

Exp. 2.1.19. Intact rabbit. Atropinized.

Rate per min.:	150	200	240	272
R.P. in sec.:	0·133	0·123	0·127	0·123

two results are shown, and it is seen that when the period changes with the rate of beating, the duration is considerably reduced and the reduction is of an entirely different order from that seen in the perfused heart. In Fig. 1 the results from three experiments are plotted, and the curve from the intact animal corresponds very closely to that found for the mammalian auricle [Lewis, Drury and Bulger, 1921]. The curves from the perfused ventricle or ventricular strip have an entirely different form.

It has been stated that in some intact animals the measurements of the refractory period were relatively constant. In animals which exhibited the usual falling curve with rise of rate attempts were made to bring about a constant measurement by altering various factors. The part played by the sympathetic nerves on the refractory period is relatively unknown, for though the work of Andrus and Carter [1930] suggests that adrenaline reduces it, their results cannot be accepted as complete proof, as the injection of adrenaline enhances the rate of

beating which in itself reduces the refractory period. In all our experiments the ventricle had been driven by rhythmic shocks, which probably stimulate sympathetic nerve endings in the muscle. Any vagal influence due to such stimulation can be prevented by the injection of atropine, but the sympathetic effect cannot be completely eliminated by any such simple method. To remove any stimulating effect of the rhythmic shocks, we have therefore made some observations in the atropinized animal in which the rate of the heart beat has been changed by heating and cooling the sinus node, and the refractory period tested in the ventricle by

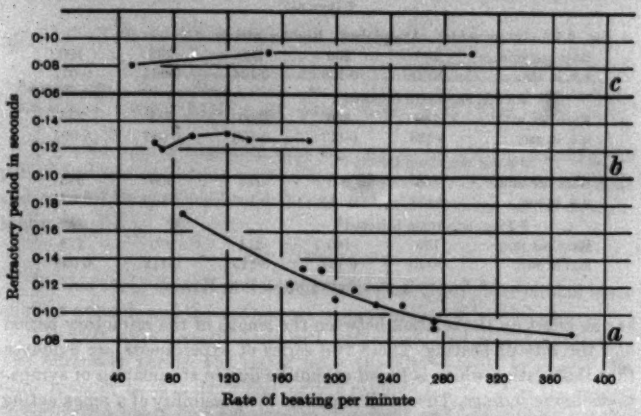

Fig. 1. Refractory period at different rates of beating of the rabbit's ventricle. *a*, intact rabbit; *b*, perfused rabbit's heart; *c*, perfused ventricular strip.

sending in occasional testing shocks through fish-hooks embedded in the epicardium. In others the heart has been driven from one point and the period tested at another distant point on the ventricular surface.

The use of these methods in the mammalian auricle has been criticized [Lewis, Drury and Bulger, 1921]. The criticism is less applicable to the ventricle where the impulse spreads rapidly and practically simultaneously through the Purkinje tissue lining the chambers, and has only to travel through a small thickness of muscle to reach the epicardial surface. In an experiment in which the sinus was cooled and heated, the period measured 0·157, 0·149 and 0·123 sec. at rates of beating of 125, 172 and 225 per min. respectively. When the period was tested at

a point distant from that at which the ventricle was driven rhythmically, it measured 0·138, 0·123 and 0·106 at rhythmic rates of 200, 230 and 300 respectively.

Although ergotoxin has only a slight paralysing effect upon the sympathetic endings in the heart [Otto, 1927] it was thought desirable to determine whether it altered the relation of the period to the rate of beating. The ergotoxin was injected in successive doses of 3·3 mg. till 10 mg. had been introduced. Although the natural heart rate decreased after each injection, the results shown in Table V indicate that this drug

TABLE V.

Exp. 2.1.8. Intact rabbit. Atropinized. Right ventricle.

Rate per min.	206	256	300	333	400
R.P. in sec.	0·121	0·109	0·104	0·094	0·077
3·3 mg. ergotoxin injected*.					
Rate per min.	180	230	250	333	375
R.P. in sec.	0·138	0·117	0·103	0·083	0·094
3·3 mg. ergotoxin injected*.					
Rate per min.	150	200	214	300	375
R.P. in sec.	0·121	0·114	0·100	0·100	0·094
3·3 mg. ergotoxin injected*.					
Rate per min.	150	188	214	250	375
R.P. in sec.	0·140	0·127	0·113	0·112	0·098

* Ergotoxin phosphate (British Drug Houses).

has no effect on the relation between the length of the refractory period and the rate of beating. These two series of experiments are evidence that the relation which is found cannot be due to stimulation of sympathetic nerve endings. There still remained the possibility of a reflex acting through the sympathetic ganglia, and to test this the ganglia were removed in several experiments. Owing to the proximity of small vessels the removal was often accompanied by a slight loss of blood, but on occasions there was no bleeding. Results typical of those given in Table VI were obtained whether hæmorrhage had occurred or not.

TABLE VI.

Exp. 2.1.18. Intact rabbit. Atropinized.

	Normal			Stellate ganglia removed				
Rate per min.	176	214	290	158	171	214	250	274
R.P. in sec.	0·144	0·118	0·106	0·143	0·140	0·137	0·131	0·127

The measurements obtained before and after removal of the ganglia show that the refractory period, after removal, remains practically constant over a considerable range of rates. A slight reduction in the period

at the higher rates of beating is still seen after removal, but this is not of the same order as in the normal animal. It would appear therefore that removal of the stellate ganglia definitely influences the relation between the rate of beating and the length of the refractory period, thus bringing about conditions similar to those found in the perfused organ.

This manœuvre, however, is not the only one which has such an influence. If 50 c.c. of warm saline is injected into the heart through the external jugular vein, the inferior vena cava being obstructed, a similar result is obtained (Table VII).

TABLE VII.

Exp. 2.1.13. Right ventricle. Intact rabbit. Atropinized.

	Normal				After saline injection				
Rate per min.	200	230	260	300	150	182	200	230	150
R.P. in sec.	0·130	0·114	0·109	0·103	0·098	0·115	0·107	0·108	0·117

It would appear therefore that the change in relation between the period and rate of beating may be brought about by both these methods and cannot be specifically associated with the removal of the sympathetic ganglia.

THE CAT'S HEART.

At this stage it seemed desirable to repeat these observations upon another animal, and the cat's heart was investigated.

The cats were anæsthetized with chloralose, and atropine injected. The refractory period was tested upon the left ventricle in exactly the same manner as that described for the rabbit's heart. After the period of the intact heart had been tested, the right and left stellate ganglia were carefully removed, together with their respective thoracic chains as far as the roots of the lungs. The heart rate was slowed by this procedure. The refractory period was again tested. The heart was then removed from the body, and perfused with Ringer's solution at a temperature of 36° C., and a pressure of about 60 cm. of water and the period measured. The values obtained from such an experiment are shown in Table VIII.

TABLE VIII.

Exp. 2.1.21 *b*. Right ventricle. Cat. Atropinized

Normal			Stellates and chains removed				Perfused					
Rate per min.:												
200	250	290	176	200	230	260	158	182	207	230	250	172
R.P. in sec.:												
0·120	0·109	0·106	0·160	0·160	0·151	0·138	0·160	0·140	0·123	0·120	0·104	0·138

It is seen that with the normal atropinized heart the period shortens as the rate of beating is raised. After removal of the stellate ganglia, and their associated thoracic chains, the period, though longer, still shortens as the rate of beating is increased, as in the preceding observation. With the perfused heart, the period is shorter, but the relation between the length and the rate of beating is undisturbed.

In the cat therefore no changes are found either after stellate removal or during perfusion, which are comparable with those seen in the rabbit's heart.

One of the main differences between the hearts of the cat and the rabbit is the thickness of the ventricular muscle. The testing shocks used have been considerably above threshold value, and it was felt that the different results obtained in the two animals might be due to the shocks reaching the endocardial surface in the perfused rabbit's heart, which they fail to do in that of the cat. To test this, the period of the perfused hearts of the cat and dog was tested upon the epicardial and endocardial surfaces. In the experiments upon the cat, the animal was anæsthetized with ether, followed by chloralose. An hour was then allowed for the elimination of the ether, after which a cannula was inserted in the carotid artery and a considerable volume of blood withdrawn. The thorax and pericardium were opened and the heart removed, and perfused in the ordinary manner. Incisions were made into the left ventricle along the septal edges from the apex to the base, and the ventricular flap drawn away from the heart by a ligature, thus exposing the endocardial surface.

The dog's ventricular strip was prepared from an animal anæsthetized with morphia and chloralose. After bleeding from the carotid artery, the thorax and pericardium were opened and a cannula inserted into a branch of the anterior descending branch of the left coronary artery. The area of muscle supplied was determined by injecting saline into the cannula and was cut away from the rest of the heart.

In both instances, fish-hook electrodes were embedded in the endocardial surface which was kept as dry as possible by draining away the perfusate by means of cotton-wool wicks. The point chosen depended upon the experimental conditions, both the septum and the endocardial surface of the ventricular wall being used. A pair of fish-hook electrodes was also embedded in the epicardium of the same ventricle, and the recording electrodes placed a short distance away. The period was measured at different rates of rhythmic stimulation, the two surfaces being tested immediately after one another. The results are shown in Table IX, and it is evident that the measurements on the epicardial surface exhibit

TABLE IX.

Exp. 2.1.28. Perfused cat's heart. Left ventricle. Atropinized.					
Rate per min.	176	200	222	250	
R.P. in sec. (epicardium)	0·138	0·131	0·109	0·101	
R.P. in sec. (endocardium)	0·110	0·110	0·096	0·092	
Exp. 2.1.29. Perfused cat's heart. Left ventricle. Atropinized.					
Rate per min.	143	176	214	260	300
R.P. in sec. (epicardium)	0·146	0·137	0·124	0·117	0·099
R.P. in sec. (endocardium)	0·127	0·136	0·134	0·123	0·113
Exp. 2.2.10. Perfused dog's left ventricular strip. Atropinized.					
Rate per min.	100	133	182	240	300
R.P. in sec. (epicardium)	0·180	0·176	0·148	0·116	0·096
R.P. in sec. (endocardium)	0·132	0·108	0·109	0·095	0·092

the usual relation to the rate of beating while those on the endocardial surface remain relatively constant. They also suggest that the period is shorter in the endocardial surface at the low rates of beating. Though this may be actually correct, as we have no evidence as to the temperatures of the two surfaces, our results offer no final proof of the relative durations of the refractory periods.

These experiments lend support to the idea that the constant period in the perfused rabbit's heart is due to the testing shocks reaching the endocardial surface, which they fail to do in the perfused cat's heart. If this explanation is correct, it should be possible to demonstrate the difference between the period of the epicardial and endocardial surfaces both in the intact rabbit and cat. The endocardium has been tested by means of special stimulating electrodes. These consist of fish-hooks which have been straightened out, and everywhere carefully insulated, except on the internal surface of the barbs themselves. Two such electrodes were thrust through the lateral wall of the left ventricle about 5 mm. apart and drawn back till the barb caught the endocardium. When these stimulators are used, the heart is driven from the endocardial surface. Fish-hook stimulators were also embedded in the epicardium, and recording electrodes placed upon the muscle close by. The period was measured at the endocardial and immediately afterwards at the epicardial surface for each rate. In Table X the measurements found in

TABLE X.

Exp. 2.2.1. Intact rabbit. Atropinized. Left ventricle.						
Rate per min.	158	200	272			
R.P. in sec. (epicardium)	0·152	0·140	0·118			
R.P. in sec. (endocardium)	0·153	0·148	0·141			
Exp. 2.2.2. Intact cat. Atropinized. Left ventricle.						
Rate per min.	142	150	167	200	230	273
R.P. in sec. (epicardium)	—	0·208	—	0·172	0·151	—
R.P. in sec. (endocardium)	0·168	—	0·177	0·167	0·163	0·164

the intact rabbit and cat are shown. The observations clearly indicate that while the measurements from the epicardium shorten as the rate is raised, those from the endocardium remain relatively constant. That constant periods are found on the epicardial surface of the perfused rabbit's heart appears to be due to the fact that the testing shocks, on account of the thin ventricular walls, reach the endocardial surface. Judging from the results upon the intact rabbit the use of whole blood as a perfusate might prevent this spread, but in our experiment in which the constant period measurements were still obtained with whole blood perfusion, the heart had first been thoroughly washed through with saline, which is sufficient to render the measurements relatively constant (see Table VII).

The endocardium is lined with a complicated network of Purkinje fibres, and it seems reasonable to conclude that this tissue is responsible for the constant period measurements. To test this point, experiments were performed on the perfused hearts of the dog and the cat in which guarded electrodes were placed beneath the free strands of Purkinje tissue (false tendons) which bridge the endocardial surface of the ventricle. In the experiments on the dog a perfused ventricular strip, as described above, was used. In the cat, the heart was perfused from the aorta, and the ventricular chambers opened. The preparation was driven rhythmically from the electrodes on the Purkinje strand and the testing shocks were sent in through the same pair of electrodes. The responses were recorded from electrodes placed upon the epicardial surface. The measurements from several experiments are given in Table XI (*a*). They show that while over considerable ranges of rate the period is relatively constant, at low rates it lengthens. The lengthening appears to us to be due to the method employed. The response to the first effective stimulus is unable to await the responsiveness of the muscle, the refractory period of which, at low rates of beating, appears to be longer than that of the Purkinje tissue[1]. If the testing shock has produced a localized effect, it may be expected to disturb the conduction of the next rhythmic response, provided this occurs quickly enough, and it is found that testing shocks which fall within the refractory period as measured by the first effective stimulus, cause the next rhythmic response to be delayed, indicating that local responses to these shocks occurred which were not conducted to the recording electrodes. For instance in Exp. 2.3.22 at rate 182, a testing shock of 0·132 delays the

[1] Further evidence that this is so will be given later as a result of the measurements of the conduction interval between the first effective testing shock and its response.

TABLE XI.

(*a*) *Exp.* 2.2.23. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	52	109	182	240
R.P. in sec.	0·174	0·171	0·194	0·171

Exp. 2.3.1. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	46	92	92	120	172	200	260	300
R.P. in sec.	0·188	0·169	0·141	0·168	0·171	0·141	0·145	0·137

Exp. 2.3.16. Cat. Perfused heart. Atropinized.

Rate per min.	150	172	200	240
R.P. in sec.	0·144	0·151	0·145	0·160

Exp. 2.3.22. Cat. Perfused heart. Atropinized.

Rate per min.	95	128	150	182	214	272
R.P. in sec.	0·184	0·174	0·174	0·144	0·134	0·134
Rate per min.	158	182	222	260	280	
R.P. in sec.	0·196	0·167	0·162	0·159	0·168	
Period tested upon muscle.						
Rate per min.	94	122	200	222	260	300
R.P. in sec.	0·202	0·195	0·171	0·166	0·146	0·125

(*b*) *Exp.* 2.4.20. Cat. Perfused heart. Atropinized.

Rate per min.	94	110	150	200	240
R.P. in sec. (ordinary method)	0·159	0·143	0·131	0·112	0·113
R.P. in sec. (new method)	0·112	0·118	0·117	0·112	0·113

next rhythmic response though it gives no recordable response. To confirm this point, an experiment was performed, using a method which detects the responses of the tissue localized around the point of stimulation [Drury and Love, 1926]. This method was used on the perfused cat's heart, and it can be seen from Table XI (*b*) that while the ordinary method gives increased measurements at low rates of beating, constant values are obtained by the new method. It can be concluded therefore that over a considerable range of rates the refractory period of the Purkinje tissue has a constant value.

THE REFRACTORY PERIOD OF VENTRICULAR MUSCLE.

In animals with a thick ventricular wall such as the dog and the cat, the period measurements of the epicardial surface exhibit the same relationship to the rate of beating as that described for the dog's auricle [Lewis, Drury and Bulger, 1921] and the frog's ventricle [Mines, 1913; Tso, 1930]. In addition, we have shown that this relationship which is found in the intact cat is not disturbed when the heart is perfused. The results in such animals can therefore be accepted as measurements of the period of ventricular muscle. Many measurements have been given in this paper for rates above 100 per min. It was thought desirable to obtain measurements at very low rates of beating in addition

TABLE XII.

Exp. 2.2.10. Dog. Perfused left ventricular strip. Atropinized.

Rate per min.	24	60	75	100	115	136	200	272
R.P. in sec.	0·199	0·196	0·197	0·192	0·196	0·165	0·130	0·128

Exp. 2.2.19. Dog. Perfused left ventricular strip. Atropinized.

Rate per min.	22	40	53	68	107	114	130	150	188	250	300
R.P. in sec.	0·172	0·165	0·176	0·166	0·143	0·146	0·137	0·127	0·114	0·112	0·104

Exp. 2.3.1. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	50	100	133	171	240
R.P. in sec.	0·190	0·195	0·185	0·171	0·151

Exp. 2.3.4. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	50	100	133	172	222	272	300
R.P. in sec.	0·215	0·210	0·206	0·180	0·152	0·144	0·123

Exp. 2.3.22. Cat. Perfused heart. Left ventricle. Atropinized.

Rate per min.	94	115	162	200	222	260	272	300
R.P. in sec.	0·202	0·207	0·188	0·172	0·166	0·146	0·145	0·136

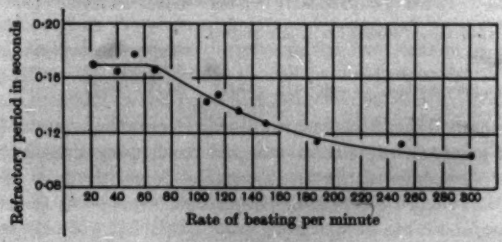

Fig. 2. Refractory period at different rates of beating of the dog's ventricular muscle (perfused strip).

to those at high rates to see whether the refractory period continues to increase, as it does between 200 and 100 per min. Such measurements cannot be obtained in the whole heart, for even if the sinus node is cooled, an A.-V. rhythm sets in which is usually about 100 per min. We have used the perfused left ventricle of the dog and the cat. The refractory period was tested upon the epicardial surface of a quiescent preparation in which very low rates of rhythmic stimulation could be used.

In Table XII the measurements of the period from several different ventricular strips are shown. These indicate that from rates of 20 to about 100 per min. the period remains relatively constant, from 100 to 200 per min. it rapidly decreases, while from 200 to 300 per min. the decrease though still definite is relatively slight. The characteristics of the curve are shown in Fig. 2 which is plotted from Exp. 2.2.19.

THE CONDUCTION RATE OF THE RESPONSE TO THE FIRST EFFECTIVE STIMULUS AT DIFFERENT RATES OF BEATING.

In many experiments the recording electrodes and stimulating electrodes have been placed about 5 mm. apart upon the epicardial surface. The interval between the first effective shock and its response is a measure of the time taken for this premature impulse to travel from the

TABLE XIII. Conduction of response to first effective stimulus at different rates of beating.

(*a*) Travelling through muscle only.

Exp. 2.2.10. Dog. Perfused left ventricular strip (atropinized).

Rate per min.	100	133	182	240	300
Conduction time in sec.	0·041	0·040	0·041	0·043	0·040

Exp. 2.3.18. Intact cat. Left ventricle (atropinized).

Rate per min.	137	162	177	220	222	240
Conduction time in sec.	0·040	0·035	0·036	0·040	0·040	0·040

Exp. 2.3.22. Intact cat. Left ventricle (atropinized).

Rate per min.	158	188	214	250
Conduction time in sec.	0·031	0·030	0·031	0·029

(*b*) Travelling from muscle to muscle, *via* Purkinje tissue.

Exp. 2.3.8. Intact cat. Left ventricle (atropinized).

Rate per min.	176	206	240	272	316
Conduction time in sec.	0·104	0·094	0·113	0·124	0·134

Exp. 2.3.16. Intact cat. Left ventricle (atropinized).

Rate per min.	146	171	200	230
Conduction time in sec.	0·085	0·084	0·114	0·122

Exp. 2.3.22. Intact cat. Left ventricle (atropinized).

Rate per min.	182	225	240	272
Conduction time in sec.	0·079	0·077	0·081	0·097

(*c*) Travelling from Purkinje to muscle tissue.

Exp. 2.2.23. Dog. Perfused left ventricular strip (atropinized). Purkinje strand stimulated.

Rate per min.	46	92	120	172	200	260	300
Conduction time in sec.	0·052	0·057	0·047	0·040	0·040	0·043	0·043

Exp. 2.3.16. Perfused cat's heart (atropinized). Purkinje strand stimulated.

Rate per min.	150	172	200	240
Conduction time in sec.	0·145	0·157	0·124	0·083

Exp. 2.3.22. Perfused cat's heart (atropinized). Purkinje strand stimulated.

Rate per min.	105	128	158	182	212	260	280
Conduction time in sec.	0·118	0·107	0·124	0·114	0·112	0·105	0·104

Exp. 2.2.10. Dog. Perfused left ventricular strip (atropinized). Endocardium stimulated.

Rate per min.	100	133	182	240	300
Conduction time in sec.	0·136	0·128	0·119	0·095	0·092

Exp. 2.1.29. Cat. Intact (atropinized). Endocardium stimulated.

Rate per min.	150	171	214	240	290
Conduction time in sec.	0·129	0·126	0·112	0·100	0·106

stimulating to the recording electrodes[1]. The interval has been measured at different rates of beating for each first effective stimulus, and the measurements between the rates of about 100–250 per min., remain practically constant (Table XIII (*a*) and Fig. 4 *a*). The impulse has two possible modes of reaching the recording contacts, it may spread laterally entirely through muscle or it may travel downwards, through muscle to the Purkinje tissue, and spreading through this, finally reach the recording contacts on the surface by coursing again through muscle. As conduction in the muscle is relatively slow compared with that in Purkinje tissue, the measurement will be of the latter path if the distance between the stimulators and recorders is more than twice the thickness

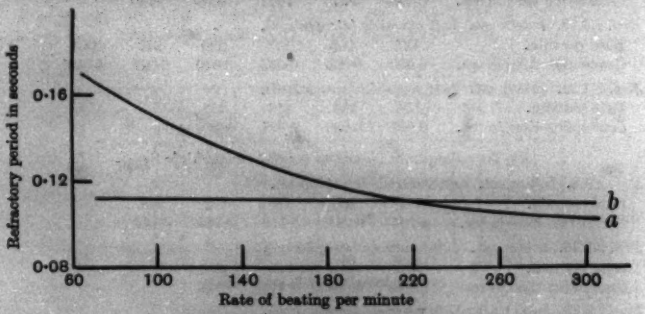

Fig. 3. Diagrammatic representation of the refractory period of (*a*) ventricular muscle, (*b*) Purkinje tissue at different rates of beating.

of the muscle wall. In our experiments, the stimulating and recording electrodes have been placed very close together so that the spread is only through muscle. It can be safely inferred that the rate of conduction through muscle immediately after the refractory period has terminated is constant at different rates of beating.

The measurements of the refractory period of the ventricular muscle and the Purkinje tissue indicate that while the former decrease as the rate of beating is raised, the latter remain relatively constant. In addition they suggest that the values of the two periods at different rates are such that the curves cross between 150 and 250 per min. This relation is diagrammatically illustrated in Fig. 3. Broadly speaking the

[1] This measurement includes the "latency of response." This is, however, in the frog extremely brief [Schellong, 1925].

refractory period of the Purkinje is shorter than that of the muscle at low rates, and longer at high rates of beating. This crossing of the curve should find expression in the conduction intervals of the response to the first effective stimulus at different rates of beating, if it is measured in such a manner that the response has traversed both types of tissue.

When it is travelling from muscle to Purkinje tissue, as the refractory period of the Purkinje tissue is shorter than that of the muscle at low rates of beating, the impulse finds the latter ready to respond. Muscle conduction has been shown to be the same at all rates of beating, so the conduction interval will remain relatively constant. It is true that as progressively lower rates of beating are tested the Purkinje tissue will have a relatively longer period of rest after the refractory period has terminated, and conduction through this tissue will therefore become progressively more rapid. On the other hand, conduction through Purkinje is extremely rapid and represents only a small portion of the total time interval, so that any such shortening might not be appreciated. At rates of beating higher than that at which the two curves cross, the muscle has a shorter period than the Purkinje, and the impulse must use up time travelling in the muscle waiting for the Purkinje tissue to become responsive. The conduction intervals will therefore lengthen, and should become longer as the two periods diverge. This assumes that the first effective impulse in the muscle will always be able to wait for the Purkinje tissue to become responsive, however long that may be. If this condition is not fulfilled, the first effective impulse will die out, and there will be a break in the curve, for the measurements will now apply to the second or even the third effective impulse. In general the measurements of the conduction of the response to the first effective stimulus travelling from muscle to Purkinje should be relatively constant till rates of about 200 per min. are reached when they should lengthen.

Experiments to test this have been made in the following manner. The heart in the intact atropinized cat[1] was driven rhythmically from fish-hook electrodes embedded in the epicardial surface of the left ventricle, while the recording contacts were placed upon the epicardial surface of the right ventricle. This method, however, involves a double passage, namely from muscle to Purkinje and Purkinje to muscle. The impulse liberated from the epicardium of the left ventricle travels from muscle to Purkinje tissue, up the left division of the bundle, over the septum, and down the right division to the muscle underlying the recording contacts on the right ventricle [Lewis and Rothschild, 1915].

[1] In order to obtain low rates of beating the sinus node was either cooled or burnt out.

The second passage, namely from the right division of the bundle to the muscle, will influence the results only at low rates of beating, for the impulse liberated will find the Purkinje tissue in the left ventricle responsive and will here suffer no delay. When it arrives at the junctions in the right ventricle, the muscle will still be irresponsive and a delay will occur. This delay will be greater the lower the rate of beating. At high rates of beating, after the curves have crossed a delay will occur in the left ventricle, as the impulse must await the responsiveness of the Purkinje tissue. As the rate of beating therefore is raised from low rhythms, the measurements will decrease and reach a minimum at the

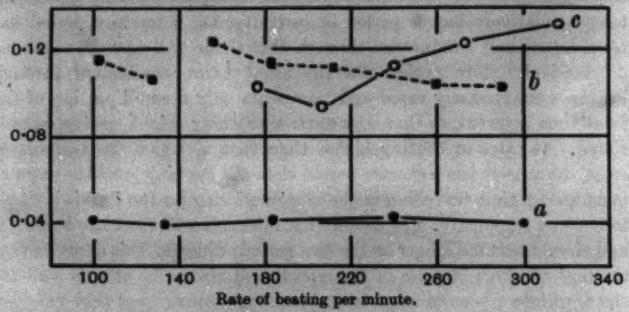

Fig. 4. Conduction of the response to the first effective stimulus. *a*, travelling through muscle; *b*, travelling from Purkinje tissue to muscle; *c*, travelling from muscle to muscle *via* Purkinje tissue.

point where the period curves cross, and then lengthen once more. The results which are given in Table XIII (*b*) and Fig. 4 *c* show that that expected result is realized.

Evidence of the delay of the passage of the impulse from Purkinje tissue to muscle at low rates of beating is provided by the measurements from the following experiments. In these the stimulators were placed upon a Purkinje strand, and the response recorded on the epicardium. In this case there is only one passage, namely from Purkinje tissue to muscle. At low rates of beating, the response in the Purkinje tissue[1] must await the responsiveness of the muscle, and will suffer a delay. After the curves have crossed no such delay will occur, and as pro-

[1] We have no evidence as to the rate at which the response to the first effective impulse liberated in Purkinje fibres travels in this tissue at different rates of beating. We have assumed that it is constant at all rates, as this is found to be the case in muscle.

gressively higher rates are tested the muscle will have a relatively longer period of rest and conduction should be quicker. A further decrease due to this factor might be expected, but at high rates the two curves diverge very slowly owing to the flattening out of the muscle curve, so that this influence cannot be very great. The measurements should therefore decrease definitely as far as the point of crossing of the curve, after which they will remain constant, or if the improvement in muscle conduction comes into play, have slightly lessened values. This result is seen in the measurements from Table XIII (*c*)[1] and Fig. 4 *b*. They show in addition that at low rates of beating an abrupt change may occur in the measurements. This is due to the response to the first effective stimulus in the Purkinje fibres being unable to await the responsiveness of the muscle, so that the measurements are now of the second or third effective stimulus. These observations are evidence that the relation between the refractory period of Purkinje tissue and muscle which was indicated by actual measurement is correct.

The diagrammatic representation of the two periods in Fig. 3 appears to us to represent, reasonably accurately, the relation as it actually exists.

The influence of diastolic filling upon the refractory period.

Tso [1930] has shown that variations in the diastolic filling of the frog's ventricle are without influence upon the refractory period provided the rate of beating is maintained constant. These results have also been found by us for the dog's auricular and ventricular muscle. The experiments upon the auricular muscle were carried out on the intact atropinized animal. After exposing the heart in the usual manner, ligatures were placed around the superior and inferior venæ cavæ. These vessels could be closed by tightening these ligatures and the pressure in the right auricle reduced. To produce raised auricular pressure, considerable volumes of saline were injected into the femoral vein. The auricular pressure was measured in some experiments by inserting a wide-bored cannula connected to a saline manometer into the superior vena cava and leading it into the auricular cavity. The diastolic filling could be increased or decreased by these methods, and as the actual measurements of the period were made immediately, it is unlikely that

[1] The measurements at high rates of beating from the experiments in which the endocardium was stimulated are not strictly comparable with those at low rates. The former may represent muscle conduction only as the refractory period of this tissue is shorter than that of Purkinje fibres at these rates.

the results were influenced by the many other changes which must accompany these procedures. The measurements were made upon the right auricular muscle, the stimulating electrodes being placed upon the body of the auricle and the recording electrodes a few millimetres away. The results (Table XIV (*a*)) show that the pressure within the auricle has no influence upon the refractory period at a constant rate of beating, and this holds for considerable variations in venous pressure.

TABLE XIV.

(*a*) *Exps.* 0.10.9, 0.10.14. Intact dog, fully atropinized. Right auricle.

Rate per min.	225	225	225	160	160	160	160
Auricular pressure in cm. of H_2O	22	16	18	13	10	21	13
R.P. in sec.	0·100	0·090	0·086	0·108	0·105	0·103	0·109

(*b*) *Exp.* 1.5.6. Dog's heart-lung preparation. Right ventricle.

Rate per min.:										
214	107	214	120	240	240	120	120	120	120	120
Output in c.c. per min.:										
250	125	125	150	150	310	310	Small	Medium	Large	Small
R.P. in sec.:										
0·14	0·16	0·14	0·17	0·14	0·14	0·15	0·19	0·17	0·17	0·17

The same result can be demonstrated in the dog's heart-lung preparation for the right ventricular muscle. The diastolic filling was changed by altering the venous pressure, and was measured in the experiment by the change in outflow from the left ventricle. The results in such an experiment are tabulated in Table XIV (*b*) and indicate that the period decreases with the rate of beating whatever the venous pressure, and remains constant at the same rate of beating though the venous pressure is changed. These experiments agree with Tso's findings for the frog's ventricle [1930]. It is evident therefore that the refractory period of the auricular and ventricular muscle in the dog is uninfluenced by diastolic filling.

It has been demonstrated in the perfused rabbit's heart that the period measured is not entirely that of ventricular muscle, but also of Purkinje tissue. This tissue is likewise uninfluenced by the initial length to which the muscle is stretched. This was shown in the whole perfused heart by inserting a bladder into the right ventricular cavity connected to a column of liquid paraffin whose height could be increased or decreased. The results (Table XV) show that the period remains constant with the same pressure, though the rate is increased, and also when the rate of beating is held constant and the pressure within the cavity considerably altered. The same result was found for the perfused strip. In this preparation we attached six threads radially which passed over

light pulleys, and on which weights were hung. By altering the weights the muscle could be subjected to different degrees of stretching. The measurements (Table XV) obtained by this method agree with those found for the whole perfused heart.

TABLE XV.

Exp. 1.2.26. Perfused rabbit's heart.

Rate per min.	150	150	150	150	300		
R.P. in sec.	0·152	0·149	0·146	0·184	0·179		
Pressure	Great	Mod.	Slight	Slight	Slight		
Rate per min.	150	150	150	150	150	150	150
R.P. in sec.	0·159	0·165	0·156	0·144	0·140	0·135	0·145
Pressure	Slight	Mod.	Great	Mod.	Slight	Mod.	Great

Exp. 1.2.20. Rabbit's heart. Left ventricular strip.

	Weights attached			No weights		Weights attached		
Rate per min.	43	129	258	43	129	43	129	258
R.P. in sec.	0·14	0·14	0·12	0·17	0·16	0·18	0·15	0·14

	No weights	Small weights	Large weights	No weights	Large weights	Small weights
Rate per min.	136	136	136	136	136	136
R.P. in sec.	0·11	0·12	0·12	0·11	0·12	0·13

The experiments indicate that the refractory period of mammalian heart muscle is uninfluenced by diastolic filling. In addition they show that that of the Purkinje tissue which is responsible for the constant period is likewise uninfluenced.

CONCLUSIONS.

The foregoing observations clearly indicate that the refractory periods of the two tissues, namely muscle and Purkinje, which make up the ventricles have very different characteristics. The period of the Purkinje tissue remains relatively constant, while that of the muscle shortens as the rate of beating is raised. Moreover, there is evidence that the relative durations of the periods is such that at low rates of beating that of the Purkinje fibre is shorter than that of muscle, while at high rates the reverse holds, the curves crossing at rates of 150–250 per min. This crossing of the curve is only seen in occasional experiments on the perfused heart, for it is impossible to maintain the conditions at the Purkinje strand identical with those at the epicardial surface, both as regards temperature and supply of perfusate. In the experiments upon the intact animal, in which both the endocardial and epicardial periods have been compared, the crossing of the curve is not seen for the reason that the testing shocks applied to the endocardium are not limited to Purkinje

tissue only, and they will always measure the shorter period. In such experiments the period measured at high rates is that of muscle, while at low rates it is that of Purkinje tissue. This was indicated in some experiments in the intact cat in which the measurements remained relatively constant till a rate of about 250 per min. was reached, when they shortened abruptly, and followed a curve very similar to that of muscle tissue. In the perfused rabbit's heart, in which we conclude that the testing shocks reach the Purkinje tissue, there has been a tendency for the measurements at high rates to be shorter than those at low rates of beating. This is again due to the fact that at low rates the measurement is that of Purkinje and at high rates that of muscle.

When, in the experiments on the cat and dog, the measurements from the endocardium are compared with those from the Purkinje strands it is seen that in the former case the values remain constant at rates at which a sudden increase is seen in the latter. The reason for the difference between the results is not clear to us, but it may well be due to the fact that nutritive condition of the free strand is not nearly so good as that of the fibres which line the endocardium.

The perfused rabbit's heart and ventricular strips give measurements which remain constant at much lower rates of beating than is the case in the hearts of the dog and the cat when the endocardium is tested. It must be concluded that in the rabbit the impulses liberated in the Purkinje fibres are better able to await the responsiveness of the muscle tissue.

The use of the perfused heart does not alter the characteristics of the period measurements of cardiac muscle, but in the rabbit owing to the thin ventricular wall[1] the shocks are liable to reach the Purkinje fibres, and this gives rise to false results. This may even occur in the intact rabbit. In animals in which the ventricular wall is thick this error does not occur, and the measurements of the muscle period have the same characteristics whether they are tested upon the intact or the perfused heart.

Summary.

1. The refractory period of the perfused rabbit's ventricle, tested on its epicardial surface, remains constant over a considerable range of rates.

2. In the perfused heart of the cat and dog, a constant period measurement is obtained when the endocardial surface or an isolated

[1] The distribution of the Purkinje fibres in the rabbit may differ from that in the cat and dog; they may reach more superficial layers.

Purkinje strand is tested, but the epicardial surface shows the usual relation between period and rate of beating.

3. It is concluded that the refractory period of Purkinje tissue remains constant irrespective of the rate of beating, and that in the perfused rabbit's heart conditions are such that the testing shocks applied to the epicardium reach the Purkinje network.

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THE ACTION OF SOME AMINES RELATED TO ADRENALINE.

I. Methoxy-phenylethylamines.

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The discovery of the composition and structure of adrenaline has led to the investigation of a large number of related compounds in which modifications of adrenaline have been brought about by alteration either of the catechol nucleus or of the methyl-amino-ethanol side chain or of both. Dakin [1905], who was one of the first to explore the actions of compounds related to adrenaline, found that catechol itself produced a rise of blood-pressure in rabbits but that the methyl ether of catechol produced no such effect. He concluded: "It appears that the catechol nucleus is essential for the production of physiologically active substances of the type of adrenaline," and "It is of importance that the hydrogen atoms of both hydroxyl groups in the catechol nucleus be un-substituted."

Later Barger and Dale [1910], in their searching examination of the factors responsible for the adrenaline type of action, showed that the rise of blood-pressure produced by catechol was due to a general stimulation of plain muscle and that catechol itself had not the specific actions of adrenaline.

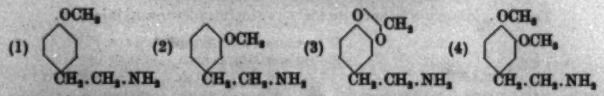
Though the methyl ether of catechol is itself inactive, it does not, therefore, follow that substitution of the hydroxy groups of the catechol nucleus in an adrenaline type of compound will also annul its activity, because catechol does not possess the adrenaline type of action. Nevertheless, it is probable that Dakin's suggestion (that, for the adrenaline type of action, it is important that the hydroxyl groups in catechol nucleus be unsubstituted) has had the effect of deterring subsequent workers from examination of compounds related to adrenaline in which this substitution has been made. Indeed, among the large number of

[1] The compounds were prepared by C. J. Virden and the pharmacological actions investigated by D. Epstein and J. A. Gunn.

substances related to adrenaline which have been investigated, we have found no records of the pharmacological examination of the compounds which are here described.

The primary object of the present investigation was to test the activity of this type of compound, and the following compounds, which were prepared in this laboratory by Dr Virden, have been investigated:

(1) *p*-methoxy-phenylethylamine.
(2) *m*-methoxy-phenylethylamine.
(3) Methylene-dioxy-phenylethylamine.
(4) Dimethoxy-phenylethylamine.



The first compound is the methylether of tyramine, the actions of which have been the subject of numerous investigations.

The effect of etherification of a phenolic-hydroxyl group on the physiological activity of a particular compound is difficult to predict. It may lead to a diminution, to an increase, or to a change in activity. In the particular group of compounds under consideration there was reason to expect that such etherification would lead to a diminution of activity, but, as we shall show, it actually led to quantitative and qualitative changes in activity that were hardly to be anticipated. Indeed we found to our surprise that the fourth compound which approximated most closely to adrenaline in structure was the farthest removed from it in physiological action.

In the investigation of the compounds mentioned we had a secondary —and from the therapeutic point of view more important—object in mind, namely, the endeavour to discover a compound which might combine some of the advantages of adrenaline and ephedrine. In the treatment of asthma, for example, adrenaline has the merits of speed and certainty of action; but has the disadvantages that its effects are transient and that it has to be given by injection. Ephedrine produces a more prolonged action and can be given by mouth, but may produce undesirable side-effects, *e.g.* prolonged rise of blood-pressure, with nausea, headache, palpitation and insomnia. The instability and brevity of action of adrenaline is chiefly due to the presence of the two phenolic-hydroxyl groups, and the comparative stability of ephedrine to their absence. The comparative diminution in absolute activity of ephedrine is not a serious dis-

advantage, as it merely means that it has to be given in a larger dose than adrenaline. It was possible that, by adopting some other method of stabilizing the nucleus, one might obtain a substance which would possess some of the attributes of ephedrine without its disadvantages; and methylation of the hydroxyl groups was an obvious method of stabilizing the nucleus and of producing a substance sufficiently resistant to chemical change to suggest that it might produce its actions when given by mouth and produce effects more enduring than those of adrenaline; provided always that this etherification did not stabilize the substance to the point of annulling physiological activity.

TABLE I. Minimum lethal dose for mice, by intraperitoneal injection.

Drug used	Wt. of mouse in g.	Dose per kg. in g.	Actual dose in g.	Result
p-methoxy-phenylethylamine (1)	30	0·3	0·009	Died in 7 minutes
	29	0·24	0·007	Died in 8 minutes
	23	0·22	0·005	Died within 30 hours
	25	0·2	0·005	Died within 2 days
	19	0·17	0·0033	Died within 2 days
	22	0·15	0·0033	Died within 3 days
	27	0·11	0·003	Recovered
m-methoxy-phenylethylamine (2)	22	0·45	0·07	Died in 8 minutes
	26	0·31	0·008	Died in 7 minutes
	35	0·23	0·008	Died in 8 minutes
	25	0·2	0·005	Recovered
	23	0·17	0·004	Recovered
Methylene-dioxy-phenylethylamine (3)	18	0·3	0·0054	Died in 11 minutes
	27	0·26	0·007	Recovered
	23	0·2	0·0046	Recovered
Dimethoxy-phenylethylamine (4)	27	0·55	0·015	Died in 18 minutes
	29	0·5	0·015	Died in 9 minutes
	38	0·42	0·016	Died in 17 minutes
	27	0·4	0·011	Recovered
	34	0·36	0·012	Recovered
	32	0·31	0·010	Recovered
	26	0·19	0·005	Recovered
p-hydroxy-phenylethylamine (tyramine) (5)	21	0·8	0·017	Died in 3 days
	23	0·65	0·015	Recovered
	26	0·5	0·013	Recovered
	24	0·33	0·008	Recovered
	22	0·23	0·005	Recovered
	25	0·16	0·004	Recovered

The present investigation seems to have gone some way towards finding such a substance. It has also revealed some unexpected difference not only between these compounds and adrenaline but between the compounds themselves. These differences concern especially actions on the central nervous system and the so-called "sympathomimetic" actions.

It will be convenient first to compare the toxicities of the compounds

and to describe briefly the general effects produced by them in the intact animal: and subsequently to discuss the actions on various organs with special reference to sympathomimetic action.

In order to obtain some idea of the relative toxicity of these compounds and of the general effects produced by them, experiments were made on mice, the amines being given by intraperitoneal injection. By this type of experiment the maximum amount of information in regard to laboratory mammals can be obtained with a limited supply of drug.

From Table I it can be seen that the approximate minimum lethal dose per kg. for mice by intraperitoneal injection is as follows:

	g.	Ratio
(1) *p*-methoxy-phenylethylamine	0·15	1·0
(2) *m*-methoxy-phenylethylamine	0·23	1·5
(3) Methylene-dioxy-phenylethylamine	0·30	2·0
(4) Dimethoxy-phenylethylamine	0·42	2·8
(5) *p*-hydroxy-phenylethylamine	0·8	5·3

Several points of importance emerge from these results, which incidentally emphasize the importance, in any comparison of a homologous series of drugs, of experiments on the intact and unanæsthetized animal. Often, when such a series of drugs is being compared, this comparison is based upon a single criterion of physiological activity (*e.g.* effect on blood-pressure), and it is at least implied that the data afforded by this single criterion give satisfactory *prima facie* evidence for their relative general activities. The fallacy of such an assumption can be illustrated by a comparison of *p*-methoxy-phenylethylamine (1) with *p*-hydroxy-phenylethylamine (5). When injected intravenously the latter has a more powerful action on the blood-pressure than the former and might therefore be said to be more active, but, when given by intraperitoneal injection in the intact animal, the former is at least four times as toxic as the latter.

Another point of interest relates to the time of death. In the case of compounds (2), (3) and (4), if the animal dies, death occurs within a few minutes, but with compounds (1) and (5) there is a more chronic form of poisoning in which the animal dies after two or three days. It may be of some significance that both these compounds (1) and (5) are para compounds.

As will be shown later, compounds (1) and (2) seem to be, quantitatively and qualitatively, indistinguishable as regards their effects on blood-pressure and on isolated organs. If, in the case of intraperitoneal injections in the intact animal, only those cases be considered in which

death occurs in a few minutes, the toxicities of both are, within experimental limits, also identical (24 : 23). On the other hand, if the animals were kept under observation for several days, it was found that the para compound is considerably more toxic than the meta compound by reason of the delayed poisoning that it produces. It is a remarkable fact that, of the two isomeric compounds which differ chemically only in the position of the methoxy group and which resemble one another otherwise so closely in their physiological action, one should produce a type of delayed poisoning from which the other seems to be entirely free.

Symptoms produced.

(1) p-*methoxy-phenylethylamine.*

The chief symptoms produced were irregular and jerky contractions of the voluntary muscles, especially of the head, with ataxia and loss of power of progression. The animals showed a characteristic type of tremor which resembled coarse shivering. When death occurred within a few minutes, it was due chiefly to respiratory failure, and was preceded by cyanosis and weak asphyxial convulsions. The heart continued to beat feebly for some minutes after respiration ceased. If the dose was just sufficient to produce delayed death, the tremors and ataxia subsided in an hour or so, after which the animal might remain apparently normal for 24 hours or more. Then a type of paralysis came on, characterized by a great fall of temperature and by extremely slow contractions of the voluntary muscles.

(2) m-*methoxy-phenylethylamine.*

With the meta compound the delayed type of poisoning did not occur, and it differed otherwise in its acute effects on the central nervous system. The chief differences were that the meta compound produced more marked acceleration of respiration and increased excitability of the spinal reflexes. While, therefore, the para compounds seemed to excite the motor cortex of the brain, the meta compound, in addition, stimulated the respiratory centre and spinal cord.

(3) *Methylene-dioxy-phenylethylamine.*

This compound produced symptoms, intermediate between those produced by compounds (1) and (2). Certain movements which were characteristic of (2) were reproduced, but there was less increase of spinal excitability than was seen with (2).

(4) *Dimethoxy-phenylethylamine.*

This compound produced quite different effects from the previous three. There were no marked signs of increase of excitability of the motor cortex of the brain or of the spinal cord. The animals became gradually paralysed and died from respiratory failure.

A full comparison of the general effects of the four compounds would require detailed and lengthy protocols. It may suffice to point out that, in this group of compounds which possess an identical ethylamine side-chain, relatively small differences in the structure of the nucleus produce considerable changes in the action of the resulting compounds on the central nervous system. Compounds (1), (2) and (3) resemble one another fairly closely, but (4) is quite different. It will be shown later that, in its other actions also, (4) produces quite different effects from (1), (2) and (3).

ACTION ON THE ISOLATED MAMMALIAN HEART.

Compounds (1), (2) *and* (3).

The hearts were perfused by means of Gunn's apparatus. It was found that compounds (1), (2) and (3) produced a typical sympathomimetic effect (augmentation and acceleration) on the cat's heart but no such effect on the heart of the rabbit. Fig. 1 shows the effect of the same concentration of *p*-methoxy-phenylethylamine (1 in 20,000) on the hearts of the cat and rabbit. The characteristic sympathomimetic effect is produced in the former, but no effect at all on the latter. The same difference in response was seen with compounds (2) and (3).

This difference in response was not merely a quantitative one, because, however high the concentrations used, the sympathomimetic action was absent in the rabbit's heart. This is unmistakably shown in Fig. 2. The upper tracing shows that even 1 in 500,000 of compound (2) produced a striking sympathomimetic action on the heart of the cat whereas a concentration fifty times as strong (lower tracing) has no effect on the rabbit's heart. No sympathomimetic action was produced on the rabbit's heart even with solutions so concentrated as 1 in 5000. Compound (3) seems to be the most active of the three on the cat's heart, a quite considerable degree of augmentation and acceleration occurring with a solution of 1 in 2,000,000.

Compound (4).

Dimethoxy-phenylethylamine was found to have no sympathomimetic effect on the heart either of the rabbit or of the cat. Fig. 3 shows the effect of a solution of 1 in 10,000 on the cat's heart. This produced slowing and depression of the heart well manifested in two minutes. When the amine solution was replaced by Locke's solution (L.S.) the heart rapidly recovered and in four minutes had almost returned to its normal rate and amplitude of beat.

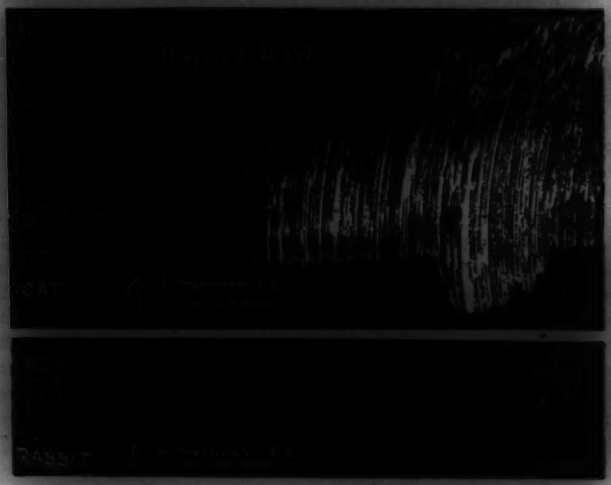


Fig. 1. Isolated perfused mammalian hearts: *p*-methoxy-phenylethylamine 1 in 20,000. Showing sympathomimetic action in the cat (upper tracing) but not in the rabbit (lower tracing).

ACTION ON THE CORONARY VESSELS.

Adrenaline is known to dilate the coronary vessels, though this effect cannot readily be demonstrated unequivocally in the perfused heart, because it is difficult to determine to what extent a change in the rate of flow is due indirectly to alterations in the heart's contractions. In most of our experiments on the perfused heart we recorded the coronary flow by Gunn's syphon recorder [Gunn, 1926]. In Fig. 2 it can be seen that compound (2) in a concentration of 1 in 500,000 produces a very great

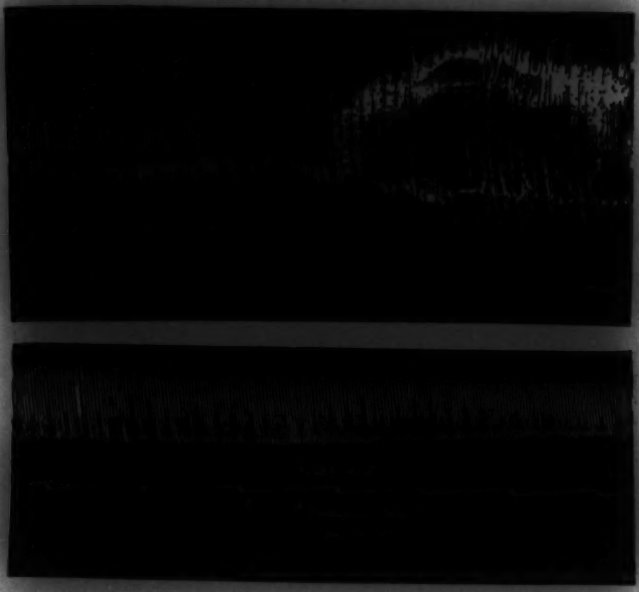


Fig. 2. Upper tracing = *a*, lower tracing = *b*. Isolated perfused mammalian hearts: showing sympathomimetic action of methylene-dioxy-phenylethylamine, 1 in 500,000, on the cat's heart (*a*), and absence of this effect even with 1 in 10,000 on the rabbit's heart (*b*). *C.F.* = coronary flow.

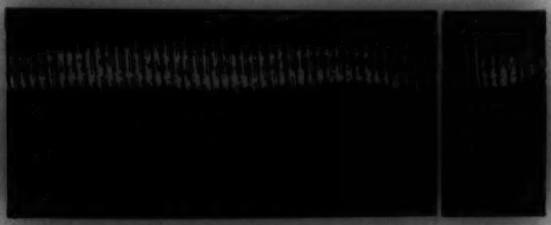


Fig. 3. Isolated perfused cat's heart: dimethoxy-phenylethylamine 1 in 10,000. Showing absence of sympathomimetic action with this compound on the cat's heart.

increase (about 500 p.c.) in the rate of flow through the coronary vessels of the cat, whereas a concentration fifty times as strong has no effect on the flow through the heart of the rabbit.

Fig. 4 shows that increased rate of coronary flow is not dependent on the changes in the heart's contractions. A solution of 1 in 200,000 of the meta compound produced an increase in the rate of flow of about 400 p.c., which could hardly be explained by the relatively slight augmentation of beat. The latter explanation was also negatived by the effect of replacing the amine solution by Locke's solution, because this produced a reduction in the coronary flow in spite of still continued and even enhanced aug-

Fig. 4. Isolated perfused cat's heart: *V.* = contractions of ventricle, *C.F.* = coronary flow, each notch representing 2 c.c. Showing sympathomimetic action on the heart, with increased flow through the coronary vessels, produced by *m*-methoxy-phenylethylamine, 1 in 200,000.

mentation of beat. The stimulant effect on the heart of the cat produced by compounds (1), (2) and especially (3) was very prolonged and often continued for many minutes after the solution was replaced by Locke's solution. The effect on the coronary vessels passed off much more rapidly. There was also, particularly with compound (3), a noticeable delay (*vide* Fig. 2 *a*) in the onset of the stimulant effect, as compared with the sudden effect of adrenaline. This delay in action was observed also in the case of the other isolated organs.

From these experiments on the isolated heart one could not evade the conclusion, which was far from expected, that compounds (1), (2) and (3) stimulated the sympathetic in the heart of the cat but not of the rabbit, while compound (4) had no sympathomimetic effect on the heart of either species. The results both on the heart itself and on the coronary vessels supported this conclusion.

Action on blood-pressure.

(*a*) *Cat.*

A large number of experiments were made on cats (decapitated) not only to determine the nature of the effects produced by the amines under consideration, but also to obtain some idea of the quantitative relationships between their activities and that of adrenaline.

Fig. 5 attempts to give an epitome of these results. The method of obtaining this tracing may possess some novelty. The original records

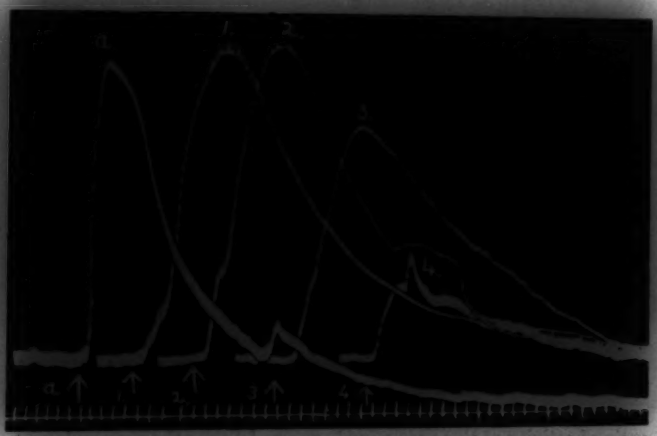


Fig. 5. Record of blood-pressure in decerebrate cat: showing comparative effects on blood-pressure of the following compounds in mg. per kg.: adrenaline 0·015 mg.; (1) *p*-methoxy-phenylethylamine 4·0 mg.; (2) *m*-methoxy-phenylethylamine 4·0 mg.; (3) methylene-dioxy-phenylethylamine 4·0 mg.; (4) dimethoxy-phenylethylamine 4·0 mg. Compounds (1), (2) and (3) resemble adrenaline in action qualitatively.

were taken on a continuously revolving drum in the usual way. The tracings giving the effect of each drug were subsequently photographed separately on half-plate negatives, but at graded distances from the left edge of the plate. The five plates were then placed one over the other and a print taken through all five. The negatives were of course all taken to the same scale. We are indebted to Mr E. G. Long for making this print according to instructions.

Table II gives the actual data. The corresponding figures for the rabbit are also given and will be referred to later.

TABLE II.

		Cat		Rabbit
Drug	Dose per kg. in mg.	Rise of blood-pressure in mm. Hg	Duration of rise in minutes	Rise of blood-pressure in mm. Hg
Adrenaline	0·015	140	2½	88
Compound (1)	4·0	145	4	20
Compound (2)	4·0	150	4	—
Compound (3)	4·0	128	7	15
Compound (4)	4·0	50	3½	30

The para and meta compounds produced, in equal doses, rises of blood-pressure almost equal in height and duration. In this respect they possess at least 1/300 of the activity of adrenaline and the effect is rather more prolonged. The methylene compound produced a smaller but more lasting rise of pressure.

Compound (4) produced a comparatively small rise of pressure with a curve different from that of the others. A second injection of this compound, of 16 mg. per kg., produced a very transient rise of pressure of 30 mm. followed by a prolonged fall.

One experiment of the type described might not necessarily give comparable quantitative results because the previous injection of one or more drugs might render the animal less sensitive to the action of subsequent injections. Many such experiments have been done, however, in which the order of drugs injected has been different and the results were not substantially modified. From several experiments the average ratio of activity of these compounds was as follows: the para and meta compound had about 1/300, and the methylene compound 1/400, of the activity of adrenaline on the blood-pressure of the cat, and the type of pressure curve produced by them was entirely compatible with the view that they stimulate the sympathetic.

As in the case of the heart and coronary vessels, the dimethoxy compound (4) did not produce a typical sympathomimetic effect. It produced in small doses a small rise of pressure.

Larger doses produced a temporary rise of pressure followed by a fall. Increase of dose beyond a certain point did not produce a further rise of pressure. In this respect also, it differed from the other three compounds. The secondary fall of blood-pressure produced in the cat by compound (4) bears at any rate a superficial resemblance to that pro-

duced by histamine. Fig. 6 (*a*) shows the preliminary rise and secondary fall of blood produced in a decapitated cat by the dimethoxy compound. This fall lasted several minutes. Six minutes later the same dose of the methylene compound was given (*b*), which produced the usual rise of pressure. Later histamine 1 mg. per kg. produced a characteristic fall of pressure (*c*).

This experiment shows that, when the main effect of (*a*) is a fall of blood-pressure, (*b*) given subsequently still produces a rise of pressure. Those compounds, therefore, act in different ways. The rise of pressure in (*a*) is due to a stimulation of arterial muscle and the subsequent fall in some ways resembles that produced by histamine (*c*).

Fig. 6. Record of blood-pressure: showing (*a*) transient rise, followed by a fall, of B.P. by dimethoxy-phenylethylamine; (*b*) rise of B.P. by methylene-dioxy-phenylethylamine; and (*c*) fall of B.P. by histamine.

(*b*) *Rabbit.*

If the hypothesis (that the first three compounds stimulate the sympathetic in the cat but not in the rabbit, while compound (4) stimulates the sympathetic in neither species) drawn from experiments on the isolated heart were correct, then it would be reasonable to expect that there would be corresponding differences in the effects of the first three compounds on the blood-pressure of the cat and rabbit. This was found to be the case. In Table II are compared the effects of similar doses per kg. on the cat and rabbit. The rabbits were anæsthetized by urethane and had both vagi cut, so as to remove cardiac inhibitory influences which are also absent in the decerebrate cat. The table shows that, under these conditions, a given dose of adrenaline produces a smaller rise of pressure in the rabbit than in the cat. It also shows a complete lack of correspondence between the effects of equivalent doses of the amines under consideration on the blood-pressure of the two species. Thus a dose of the

para compound (4 mg. per kg.) which, in the cat, caused a rise of blood-pressure equal to that produced by 0·015 mg. of adrenaline, caused, in the rabbit, less than 25 p.c. of the rise produced by the same dose of adrenaline. Moreover a dose of the dimethoxy compound, which caused in the cat a rise of pressure of one-third of that produced by the para compound, caused a greater rise than the latter on the blood-pressure of the rabbit.

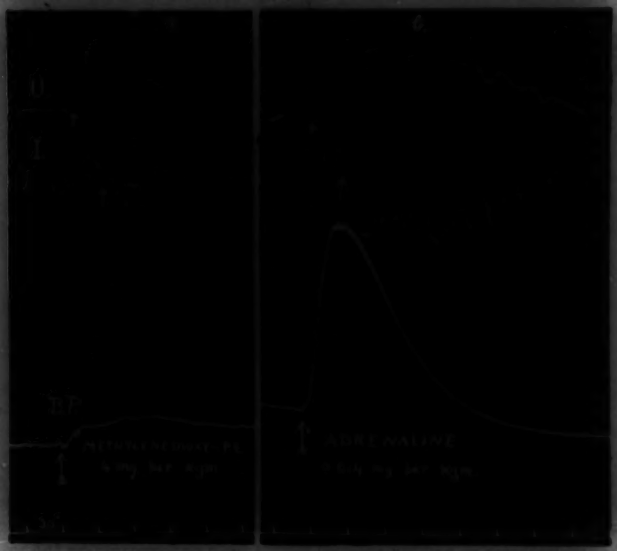


Fig. 7. Records of uterine movements (*U.*), intestinal movements (*I.*), contractions recorded upwards, and blood-pressure (*B.P.*) in a rabbit, anæsthetized by urethane (vagi cut). Showing (*a*) that methylene-dioxy-phenylethylamine does not produce sympathomimetic effects in the rabbit. The intestine is not relaxed or the B.P. raised as by adrenaline (*b*).

In the case of all four compounds, no greater rise of pressure was produced by increasing the dose. The rise of pressure was relatively insignificant and bore little resemblance to a sympathomimetic effect. This is illustrated in Fig. 7. In this experiment the same doses were given as in Figs. 5 and 8, but the effect of the methylene compound in the rabbit is inconspicuous as compared with the effect in the cat. This difference is

also shown by comparison with the same dose of adrenaline in each species.

Experiments on blood-pressure, like those on the isolated heart, suggest, therefore, that the first three compounds stimulate the sympathetic in the cat but not in the rabbit. In the rabbit they produce a slight rise of blood-pressure, such as might be produced by a weak stimulation of arterial muscle. The latter type of effect is produced by the dimethoxy compound in both species.

Additional evidence as to the presence or absence of sympathomimetic action was obtained from experiments on other types of smooth muscle, viz. uterus, intestine, bronchi and pupil.

Uterus.

The uterus affords a convenient test for sympathomimetic action as the nature of its response to sympathetic stimulation varies with different species. Experiments were done on the uterus both *in situ* and isolated.

In the former case the anæsthetized animal was immersed up to the thorax in a saline bath at body temperature; the abdomen was opened, the uterus at some point near the vaginal end was attached to a rigid rod, and a hook, inserted in the uterus nearer by an inch or more to the ovarian end, was attached by a thread to a lever which recorded the uterine movements. In some experiments the movements of the intestine were similarly and simultaneously recorded. A record was also taken of the blood-pressure.

Cat. In the anæsthetized or decapitated animal, compounds (1), (2) and (3) produced a relaxation, and inhibition of movement, of the non-pregnant uterus, identical with that produced by adrenaline. This is illustrated in Fig. 8.

The isolated non-pregnant uterus was also inhibited by these compounds, in weaker concentrations (*e.g.* Fig. 9 *a*). Stronger solutions, however, sometimes caused contraction of a non-pregnant uterus which was inhibited in the usual manner by adrenaline. There was considerable variation in different uteri as to the concentration which produced relaxation or contraction.

Compound (4) produced contraction of the isolated non-pregnant cat's uterus even when all other three compounds caused relaxation. This is shown in Fig. 9 *b*. In this experiment compounds (1), (2) and (3) produced relaxation of the uterus in all concentrations up to 1 in 10,000. The

Fig. 8. Record of intestinal movements (*I.*), uterine movements (*U.*), contraction recorded upwards—and blood-pressure (*B.P.*) in a decapitated cat. Showing typical sympathomimetic effects, relaxation of non-pregnant uterus and of intestine and rise of blood-pressure. Contrast the effects in the rabbit, Fig. 7.

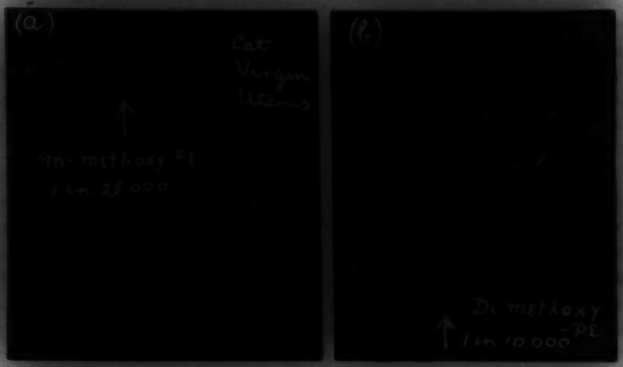


Fig. 9. Isolated virgin cat's uterus. Showing relaxation (sympathomimetic) action of *m*-methoxy-phenylethylamine, and (*b*) contraction (direct stimulation of muscle) by dimethoxy-phenylethylamine.

dimethoxy compound, however, produced a slight contraction with 1 in 25,000 and a marked contraction with 1 in 10,000.

The most obvious conclusion, and one which is supported by other evidence, to be drawn from experiments on the cat's uterus is that the first three compounds have a double action, (*a*) a stimulation of the sympathetic, which is shown in the intact animal and by weaker concentrations on the isolated organ, and (*b*) a direct stimulation of the uterine muscle, especially produced by higher concentrations, which marks the inhibitory effect of sympathetic stimulation. Factor (*a*) is weak or absent in the case of compound (4), which only exhibits the stimulant action on muscle.

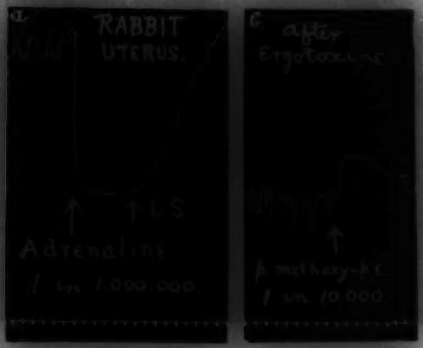


Fig. 10. Isolated rabbit's uterus. Ergotoxine, previously added, converted a motor effect of adrenaline into an inhibitor effect (*a*), but *p*-methoxy-phenylethylamine (*b*) still produced a motor effect (direct stimulation of muscle).

Rabbit. All four compounds stimulated the uterus *in situ* or isolated, of the rabbit (*vide* Fig. 7). As in this animal the effects of sympathetic stimulation and of direct stimulation of uterine muscle are identical, a motor response might be due to either action. A differentiation between the two possible actions could, however, be made by means of ergotoxine. In the experiment which is illustrated in Fig. 10, the isolated rabbit's uterus responded to adrenaline, 1 in 1,000,000, by a powerful contraction. Ergotoxine, 1 in 100,000, was then added to the bath and, subsequently, the same concentration of adrenaline produced a marked relaxation of the uterus (*a*). According to the accepted explanation, ergotoxine had paralysed the motor sympathetic terminations of the uterus. But the

para compound, as shown in the figure, still produced a pronounced contraction of the uterus (*b*). This contraction must, therefore, have been due to a direct stimulation of the uterine muscle.

Guinea-pig. The uterus of the guinea-pig, whether pregnant or non-pregnant, is relaxed by sympathetic stimulation. All four compounds, however, in whatever concentrations they were employed, caused contraction of the guinea-pig's uterus. This is shown in Fig. 11, where both

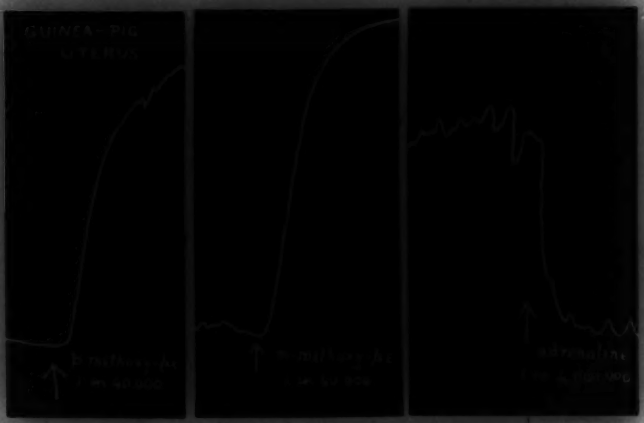


Fig. 11. Isolated guinea-pig's uterus. Showing difference in action between methoxy-phenylethylamines (direct stimulation of muscle) and adrenaline (stimulation of sympathetic).

para and meta compounds in a concentration of 1 in 40,000 caused contraction of the uterus, while adrenaline 1 in 4,000,000 caused the usual inhibition.

Rat. The uterus of the rat, which is also inhibited by adrenaline, was stimulated by all four compounds.

It is evident from these experiments that, in rodents, all four compounds have a stimulant action directly on uterine muscle and that there is no evidence of true sympathomimetic action with any of them.

Intestine.

The intestine, isolated or *in situ*, of the *cat* was inhibited by compounds (1), (2) or (3), as is illustrated in Fig. 8. Strong solutions, *e.g.* 1 in 5000, sometimes produced some increase of tonus of the exsected gut. The action on intestinal muscle, therefore, corresponds with that on the uterus, *i.e.* the effect of weaker concentrations is sympathomimetic, but a direct stimulant action on muscle may occur with strong solutions. In the *rabbit* or *guinea-pig*, however, the effect of all four compounds was to increase the tone or movements of the gut. A slight effect of this nature is seen in Fig. 7 *a*, which contrasts with the pronounced relaxation of the gut produced in the cat (Fig. 8). Larger doses produced a powerful contraction of the rabbit's intestine. The inhibitory effect of adrenaline itself on the intestine muscle is also more pronounced in the cat than in the rabbit.

Bronchi.

Experiments were made on isolated bronchial and tracheal rings. The results were of the same type as had been found with other forms of smooth muscle. In the cat compounds (1), (2) and (3), but not (4), caused a relaxation of the bronchi, but in the rings of the rabbit they failed to produce this effect.

As one of the original objects of this research was to attempt to discover a compound which would have the same action on the bronchi as adrenaline or ephedrine but would be intermediate between these two in duration of effect, a typical experiment may be described in greater detail.

As has been found by previous investigators, experiments on the bronchi, exsected or *in situ*, may fail to reveal a sympathomimetic effect (dilator) owing to the muscle of the bronchi being already in a state of maximum relaxation. We found, as others have done, that it was necessary first to give some drug which would produce a degree of bronchial constriction in order that a dilator effect could manifest itself. For this purpose we used arecoline in low concentrations.

Fig. 12 illustrates one such experiment. Arecoline, 1 in 3,000,000, was first applied and produced a marked contraction. Methylene-dioxyphenylethylamine 1 in 10,000 was then added to the bath and, in spite of the continued action of arecoline, produced a rapid and marked relaxation. The alkaloidal solutions were then replaced by Locke's solution and, after an interval, the experiment was repeated with the substitution of

ephedrine for the methylene compound. The curves of the two experiments are remarkably similar and they suggest that, so far as the cat is concerned, the methylene compound acts on the bronchi very similarly to ephedrine.

Fig. 12. Isolated bronchial rings of cat. Showing (a) relaxation by methylene-dioxyphenylethylamine of bronchi, previously constricted by arecoline. This compound acts like ephedrine (b).

Pupil.

In the cat, sympathetic stimulation, or injection of adrenaline, produces, among other effects on the eye, dilatation of the pupil and withdrawal of the nictitating membrane. Compounds (2), (3) and (4) were tested on a cat, anæsthetized with ether, to see whether they produced these effects. Compounds (2) and (3) in doses of 4 mg. per kg. produced withdrawal of nictitating membrane and wide dilatation of the pupil, but the same dose of compound (4) produced neither of these effects. This affords further confirmation for the view that compound (4) has no sympathomimetic action in the cat, corresponding to that produced by the other three compounds.

Table III gives a summary of the actions of the four compounds. It shows that in the cat the first three compounds reproduce with complete fidelity both the motor and inhibitor effects of adrenaline. In rodents, however, they produce effects which do not correspond to those of adrenaline. Especially the characteristic cardiac effects are absent and the inhibitory effects on smooth muscle are absent or replaced by stimulation. The effects in rodents are characteristic of a direct stimulation of smooth muscle.

Compound (4), on the other hand, has actions which do not correspond

with those of adrenaline even in the cat, but are typical of a stimulation of smooth muscle in all animals.

TABLE III. Comparison of action of different compounds on different tissues in different species.

[+ = contraction; − = relaxation; 0 = no effect in equivalent doses.]

	Adrenaline	Compounds (1), (2) and (3)	Compound (4)
Cat:			
Blood-pressure	+ +	+ +	+
Heart	+ +	+ +	0
Coronaries	−	−	0
Uterus (n.p.)	−	−	+
Intestine	−	−	+
Bronchi	−	−	0
Pupil	−	−	0
Rabbit:			
Blood-pressure	+ +	+	+
Heart	+ +	0	0
Uterus	+	+	+
Intestine	−	+	+
Bronchi	−	0	0
Guinea-pig and rat:			
Uterus	−	+	+
Intestine	−	+	+

DISCUSSION.

The results of these experiments seem clear-cut and allow certain conclusions as to the relation between chemical constitution and physiological action in the particular group of compounds under consideration.

Methylation of phenolic hydroxy groups in phenylethylamine compounds modifies the actions of the resulting compound on the central nervous system, on the sympathetic nervous system, and on involuntary muscle, in connection with which the following points may be of some general interest.

Central nervous system.

Methylation of a single —OH group in the para position changes the action from depressant to convulsant. A similar type of change has been described by Gunn and his co-workers in connection with the harmine alkaloids, for whereas harmol and harmalol, which possess an —OH group, are non-convulsant, harmine and harmaline, which differ from harmol and harmalol only in the methylation of this —OH group, are both convulsant [Gunn and MacKeith, 1931].

The meta-methoxy- and methylene-dioxy compounds also stimulate the central nervous system, but the action of the dimethoxy compound

is almost purely depressant. A scrutiny of the effect of methylation of hydroxyl groups (as a single change) on the action of different alkaloids on the central nervous system may, if sufficient data be obtained, lead to the possibility of some general rule being applicable to the physiological effect of this change in chemical constitution. In the meantime it is clear that relatively slight changes in the benzene nucleus of this type of compound, without alteration of the ethylamine side chain, can effect conspicuous alterations in the action on the central nervous system.

Methylation of an —OH group does not necessarily lead to diminution of general physiological activity for *p*-methoxy-phenylethylamine is, by intraperitoneal injection, five times as toxic as *p*-hydroxy-phenylethylamine.

"Sympathomimetic action."

The investigation of the actions of these compounds on the sympathetic system has led to some unexpected results. Para- and meta-methoxy-, and methylene-dioxy-, phenylethylamines all exert typical sympathomimetic actions on the cat but not on the rabbit, guinea-pig or rat. Many amines related to adrenaline produce sympathomimetic effects; but, as a rule, with greater variation from the structure of adrenaline, there occurs greater deviation from consistent or complete duplication of the effects of sympathetic stimulation. Thus, of the amines which have been more completely investigated, tyramine and ephedrine produce many effects which are definitely "sympathomimetic" but produce other effects on smooth muscle which cannot be explained simply by stimulation of sympathetic terminations. We hope in a future paper to reconsider some of the actions of tyramine and ephedrine in the light of the results obtained with the compounds dealt with in this paper. It may be suggested, however, that hitherto not sufficient attention has been paid to differences in reaction due to differences in the species of animal used. It has been usual to state that an amine has sympathomimetic actions on one organ and not on another. It would seem *a priori* more probable, however, that the "receptor" substance for any particular amine should be either present or absent in all the organs of a particular species of animal than that it should be present in one organ, and absent in another organ, of the same species of animal. In other words, it would seem more reasonable to expect that, if a substance stimulates the sympathetic terminations at all, it would stimulate them throughout in the same animal. This certainly seems to be true of the first three compounds in our series. Any one of them could (though they would be less active)

act as a sympathetic hormone in the cat but not in rodents. Adrenaline, on the other hand, seems to be an almost universal sympathetic stimulant for all species of mammals. It is, however, possible, and the possibility has not perhaps yet been excluded, that the adrenal hormone may not be in all species of animals adrenaline itself but some near relative of it.

That dimethoxy-phenylethylamine should fail to display sympathomimetic action even in the cat is a surprising result, especially in view of the fact that adrenaline has two —OH groups in the same position as the two methoxy groups in our compound. Some experiments, recently made in this laboratory with trimethoxy-phenylethylamine (mescaline) have shown that it also is devoid of sympathomimetic action. In their investigation of the amines allied to adrenaline, Barger and Dale showed that the optimum condition of the nucleus for sympathetic action was provided by two hydroxyl groups in the benzene nucleus, but that a less degree of sympathetic activity was shown by mono- and tri-hydroxy-phenylethylamines. On the other hand, sympathomimetic activity is only shown by mono-methoxy compounds, and not at all by di- or tri-methoxy compounds. One cannot at the moment hazard any plausible conjecture as to why this should be.

Direct action on smooth muscle.

All the compounds of our series stimulate smooth muscle in rodents. In the case of the first three compounds, this action is, in the cat, usually masked by sympathetic stimulation. The fourth compound is the most powerful direct stimulant of smooth muscle, and this is its only peripheral action even in the cat. In some respects it recalls the action of histamine.

Summary.

The physiological actions of (1) *p*-methoxy-phenylethylamine, (2) *m*-methoxy-phenylethylamine, (3) methylene-dioxy-phenylethylamine, and (4) dimethoxy-phenylethylamine have been investigated.

The minimum lethal dose per kg. by intraperitoneal injection for mice is approximately (1) 0·15 g., (2) 0·23 g., (3) 0·30 g. and (4) 0·42 g.

The first three stimulate, the fourth depresses, the central nervous system.

The first three stimulate the sympathetic terminations in the cat but not in rodents; the fourth has no sympathomimetic action in any of these animals.

In the decerebrate cat, compounds (1) and (2) have about 1/300, compound (3) about 1/400, of the pressor activity of adrenaline.

All four compounds have a direct stimulant action on smooth muscle in rodents.

Methylene-dioxy-phenylethylamine (3) is, in duration of effect, intermediate between adrenaline and ephedrine, and may be worthy of a clinical trial in the treatment of asthma.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

IV. Changes in the adrenal gland of the female rat associated with the œstrous cycle.

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There are many isolated bits of evidence that point towards an intimate relationship between the adrenal cortex and the reproductive system. To review the entire literature relating to the subject is too large a task to be undertaken here. The two most suggestive types of evidence are the presence of abnormalities in secondary sex characters in cases of tumours of the adrenal cortex [Glynn, 1921, and others]; and the changes in the adrenal gland associated with various phases of reproduction. The present paper treats of the latter.

Hypertrophy of the cortex has been found associated with pregnancy in guinea-pigs [Castaldi, 1922; Kolde, 1913; Guieyesse, 1899; Kolmer, 1912] and its occurrence in rabbits [Kolde, 1913; Stilling, 1898] and in rats [Herring, 1920; Donaldson, 1924] has been denied. In mice there is diminution of the size of the adrenal, but hypertrophy of the zona glomerulosa [Masui and Tamura, 1926; Tamura, 1926]. The hypertrophy in guinea-pigs has been found associated with mitoses in the cortex and with enlargement of the cells in the zona fasciculata [Kolmer, 1912; Guieyesse, 1899; Castaldi, 1922]. It continues through lactation [Verdozzi, 1914]. A similar hypertrophy has been reported in spayed and castrated animals by Schenk [1910], Kolmer [1912], Kolde [1913], and others. Hatai [1913], working on rats, reported a decrease in the female and an increase in the male adrenal after removal of the gonads.

All observers agree on one point, and that is that the adrenal weight varies greatly in normal animals. According to Donaldson [1915] the only other organ weights in rats weighing 150 g. which vary to the same degree are the gonads. Boycott and Kellaway [1924] suggest that this variability indicates that form and function are less closely related

here than in most organs. However, we are as yet unable to name the function of the cortex and are therefore at a loss to say whether hypertrophy means decreased function as in the thyroid or increased function as in most organs. Marassini [1906] noted a greater variability in the female, and many workers have observed a larger adrenal in the female than in the male.

Enlargement of the adrenal in connection with ovulation has been noted in frogs [Stilling, 1898], moles [Kolmer, 1918], and in pigeons [Riddle, 1923]. In all of these animals the reproductive season is limited to early spring. The first two undergo other marked physiological changes at that time, so that a direct correlation between the adrenal and ovulation as pointed out by Stilling is not clear. The only animal having a more frequent period of œstrus and one uncomplicated by other factors which has been studied is the mouse. Masui and Tamura [1926] reported enlargement of the adrenal cortex during œstrus in the mouse, but give very few data. Howard-Miller [1927] and Deanesley [1928] were unable to confirm it.

Many workers have investigated the effect of bilateral adrenalectomy on the œstrous cycle. The results have been variable, but the majority find a lengthening of the cycle in the adrenalectomized animals which do not have an abundance of accessories. The recent paper by Martin [1932] reviews the previous ones. That this effect is evidence of a specific cortico-gonadal relationship is unproven, as the œstrous cycle is known to be affected by any procedure which severely affects the well-being of the entire organism.

The present work is based on the probability that if adrenal changes can be found in pregnant and castrated animals they may also be associated with changes in the œstrous cycle.

The one property of the adrenal gland which can most readily be noted objectively and treated statistically is its weight, and it was felt that if a definite change in weight were found, one might expect to find also some change in the size or number of cells. Previous data obtained on adrenal weights in male rats convinced us that the relative weight was more constant, and therefore more significant than the actual weight at any given age, and it was therefore used. The rat was selected as the experimental animal because it has a regular cycle unrelated to season and because of the large numbers of animals required in obtaining reliable statistical data on so variable a thing as the adrenal weight.

Technique.

In all, 190 female virgin rats of the same breed were used, and all but a few of Series I were bred in the laboratory.

The first series of rats used in this experiment and reported in Tables I and II included 127 animals. The rats were some of those used in an experiment on the effect of thymectomy on the age of puberty. In 20 of those reported in Table I the smears were not made over a long enough period to be suitable for the analysis in Table II. The adrenals, and in some cases the thyroid and pituitary, were weighed, and it was shown that thymectomy had no effect on the weights of these organs [Andersen, 1932]. The data on these thymectomized rats are therefore included. All rats having any evidence of ear or lung infections at autopsy were carefully excluded, as these are known to affect the adrenal weights [Donaldson]. The rats were given a standard synthetic diet, *ad lib.* [Andersen, 1932], with due regard to good light, cage space, and water. Vaginal smears were made daily, beginning with the day on which the vagina opened.

Inspection of the figures obtained from these rats and grouped according to vaginal smears on the day of death showed that although there was a definite difference in the mean absolute and relative adrenal weights at different stages of the œstrous cycle, the individual variation was great, especially during the late post-œstrus and diœstrus phases. The rats with irregular cycles or cycles 6 days or more in length were then placed in a separate group and the remainder were regrouped with relation to the number of days after the first appearance of abundant cornified cells (4+) in the vaginal smear. The rats showing nucleated epithelial cells were included in the "pro-œstrus" group as before. The day after this nearly all the rats had a vaginal smear showing "4+" cornified cells, and this was termed the day of œstrus. On the two days after œstrus the smear varied greatly: there were cornified cells, non-nucleated epithelial cells or leucocytes in varying amounts. The animals were grouped according to the number of days after œstrus instead of by the smear. The rats killed during late diœstrus, when the smear was still of the diœstrus type but the uterus showed early œstrus changes, were assigned to a separate group. The resulting figures gave a much sharper difference and this plan was subsequently followed. The grouping is therefore as follows:

(1) Pro-œstrus, smear showing epithelial cells, uterus much enlarged and filled with fluid.

(2) Œstrus, the first appearance of an abundance of cornified cells, uterus enlarged but containing less fluid.

(3) The day after (2), smear usually showing 4+ cornified cells but sometimes non-nucleated epithelial cells or leucocytes, or any combination of these; uterus rather smaller and contains no fluid.

(4) The day after (3) with a smear showing a few cornified cells or leucocytes; uterus quite small.

(5) Two days after (4). This stage was included only when the rat had regular cycles of 5 or 6 days in length. When the cycles were longer the rats were excluded from this series and when shorter the data were placed under (1). It was therefore necessary to know the usual length of the cycle for each rat, which was fairly constant.

(6) The day before expected œstrus, smear dioestrus; uterus shows early œstrus changes.

Since some of these stages are so short as to be missed in smearing only once daily, the 53 rats of our second series and the 20 rats of our third series were smeared every 8 hours for a period covering at least three complete cycles. The hours chosen were 9 a.m., 5 p.m., and 1 a.m. The rats were killed according to the classification of œstrus given above. The pro-œstrus group was killed immediately after the first appearance of large numbers of nucleated epithelial cells. The "œstrus" group was killed after the first smear, in which many cornified cells were found, usually with many nucleated epithelial cells still present. The other group was killed at a given number of hours after "œstrus": day after "œstrus," after 24 hours; second day after "œstrus," after 48 hours; dioestrus, 60–72 hours; day preceding expected œstrus, at any period after 72 hours, when the uterus showed early œstrus changes and the smear was still dioestrus.

These rats were of the same breed and received the same care as the first series, with the addition of 5 p.c. of dried yeast to the diet, which had no effect on the mean weight of either the body or the endocrine glands which were weighed. The results obtained from the greater frequency of smearing are only a little more clear-cut than in the first series, probably because the mean adrenal weight changes gradually during the cycle. In this second series the rats on the whole were a little older and heavier, but the majority in both series was between 80 and 140 days of age with the actual range 68–170 days. Four rats are added which had had no cycles for many weeks: they were all 8 months old and are not included in any of the other data. A third series included ten rats on a different but adequate diet, which contained 20 p.c. of fat. The

adrenals were serially sectioned and used for data on the cortex-medulla ratio. The weights of the adrenals in Series III are not included in the figures for adrenal weights at œstrus and dicestrus, but they correspond to them quite closely.

The animals were killed with chloroform, and autopsied at once. The desired organs were quickly dissected clean of connective tissue and fat and placed at once in a closed weighing bottle. The lungs and temporal bones were examined for evidences of infection. The organs were then weighed at once to 0·1 mg. It is believed that the error in dissection and weighing of the adrenals and pituitaries is not over 0·5 mg. The error is much greater in the thyroid weights because the outlines of the gland are not clear-cut. Moreover, the parathyroids are embedded in the thyroids and the weight of the thyroid includes them also. The organs were removed from the weighing bottle one by one and the bottle was weighed after the removal of each. In the first series the adrenal was weighed in each case and the thyroid and pituitary in some cases. In the second series the pituitary, thyroid, adrenal, and thymus were weighed. The ovaries and uterus were examined and preserved, but not weighed. After weighing, the organs were placed in Zenker's fluid, except that in some cases one adrenal was placed in 10 p.c. formalin for fat stains.

In a group of 44 rats in the first series the ovaries, uterus, Fallopian tubes, pituitary, thyroid, and adrenal were sectioned. In a group of 16 rats of the second series these organs were serially sectioned. They were stained with hæmatoxylin and eosin. In the latter group frozen sections of one adrenal were made and stained for fat with Scharlach R and Nile blue sulphate. The data on the pituitary and thyroid changes will be presented in a subsequent paper.

In the ten rats of the third series both adrenals were serially sectioned at 5μ and every tenth section was mounted. The sections were stained with hæmatoxylin and eosin and tracings of the outlines of the entire adrenal and the medulla were made with a projection apparatus at a magnification of $\times 40$. These tracings were measured with a planimeter and the volume of the entire adrenal was calculated from the following formula:

$$\text{volume in cmm.} = \frac{\Sigma \text{ planimeter readings} \times 50\mu \text{ (thickness of section)}}{0{\cdot}2 \text{ (factor of planimeter)} \times 40 \text{ (magnification)}}$$
$$= \Sigma \text{ planimeter readings} \times 0{\cdot}625.$$

The volume of the cortex was obtained by subtracting the medulla from the total volume.

In each case the probable error of the mean is calculated from the formula:

$$\text{P.E.} = 2/3\sqrt{\frac{\Sigma d^2}{N}}.$$

The variations of the weight of the adrenal gland in relation to the œstrous cycle.

The first series of 127 normal virgin rats, when grouped according to the presence in the vaginal smear of epithelial cells, cornified cells, leucocytes or no cells at all, gave the figures for the mean relative weight at these various stages which are shown in Table I. The difference be-

Table I. The mean relative weight of the adrenals in various stages of the œstrous cycle grouped according to the type of vaginal smear on the day of death.

	Entire series		Rats with cycles over 6 days in length	
Stage of cycle	No.	Adrenals mg./kg.	No.	Adrenals mg./kg.
Pro-œstrus	8	250	1	193
Œstrus	32	263	4	235
Post-œstrus	29	240	7	236
Dioestrus	58	237	12	206
Entire series ...	127	246	24	219

tween the mean relative weight at œstrus and at dioestrus was not impressive, but in view of the number of animals represented it invited further investigation.

Inspection of the individual figures showed that many of the low figures were in rats having irregular cycles. After first eliminating the

Table II. The mean relative weight of the adrenals grouped according to the number of days after œstrus as well as by smear. Rats with long or irregular cycles are excluded, age 68–150 days, weight 110–182 g.

		Grouped by vaginal smear		Grouped by No. of days after œstrus	
Stage of cycle	Vaginal smear	No.	Adrenals mg./kg.	No.	Adrenals mg./kg.
I. Pro-œstrus	Nucleated E.C.	7	260	7	260±26
II. Œstrus	First day 4+ C.C.	19	283	19	283±28
III. Post-œstrus	Second day of 4+ C.C.	4	239	15	248±24
Day after œstrus	W.B.C. or non-nucleated E.C.	11	251		
IV. Dioestrus—2 days after œstrus	Varies: 4+ C.C., W.B.C. or no cells	10	234	29	233±22
V. Dioestrus—3 days after œstrus	No cells	19	233		
VI. Day before expected œstrus	Very few or no cells Uterus œstrous	13	269	13	269±25
Entire series		83		83	

rats on which records were inadequate, the rats having irregular cycles were separated off and the adrenal weights averaged. The resulting figures left no doubt that this group had a lower mean relative adrenal weight than the group as a whole (Table II). Further inspection of the remaining data showed that in many cases the rats killed on the first and second days after œstrus had no cells in the smear while others had 4+ cornified cells or leucocytes. It seemed possible that the time after œstrus was a more important factor than the type of cells in the vaginal smear. The data were regrouped as described above with the results given in Table II. The figure for œstrus is considerably increased, while that for diœstrus is lowered a little. We conclude on the basis of this finding that changes in adrenal weight with the phases of œstrus are more closely correlated with the number of days after œstrus than with the smear on the day of death.

The weight of the adrenal in relation to body weight.

The figures in Table II show that there is a greater variation in the relative adrenal weight at œstrus than at any other period, and a less variation at diœstrus. This seems strange in view of the fact that the criteria for œstrus are more sharply defined than those of diœstrus. Moreover, it happened that one group of 25 animals in the second series on which we attempted to confirm the conclusions drawn from the first series were somewhat older and heavier than those in the first group. They ranged from 100 to 150 days in age and from 150 to 225 g. in weight. In this series the œstrus adrenal was surprisingly low and the diœstrus weight was a little lower than that in Series I. We were therefore led to collect all of our data on œstrous rats and arrange them by age and weight to see if there were a definite correlation. This correlation was found as shown in Tables III and IV and Fig. 1. The relative adrenal

Table III. Rats killed at œstrus grouped by age.

Age (days)	Mean weight (g.)	No.	Adrenals	
			mg.	mg./kg.
Under 80	137	3	45·4	329
81– 90	147	3	40·7	295
91–100	163·1	8	44·6	279
101–110	162·6	7	42·7	269
111–120	159·5	4	42·0	224
121–130	198·3	3	48·0	245
131–140	195·0	3	42·2	218
141–150	188·6	5	48·7	264
151–160	—	0	—	—
161–170	219·8	4	51·9	236
		Total 40		

TABLE IV. Rats killed at œstrus grouped by weight.

Weight in g.	No.	Mean age (days)	Adrenals mg.	Adrenals mg./kg.
120–139	4	81·8	41·3	318 ±18
140–159	10	101·4	43·0	287 ±28
160–179	13	109·2	44·0	257 ±14
180–199	4	131·2	49·5	258 ±27
200–219	7	129·7	47·7	225 ±20
220–249	2	166·0	55·0	235 ±13
Total	40			

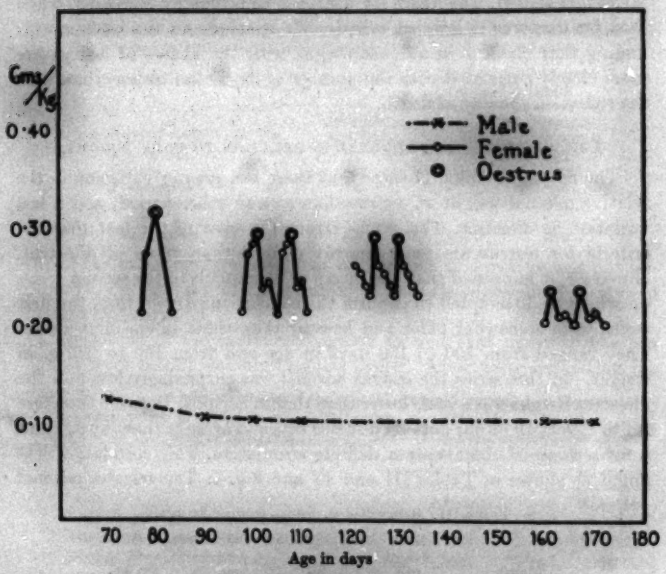


Fig. 1. The relative weight of the adrenal gland at various ages in mature male and female rats.

weight decreases progressively as the weight of the rat increases. This relationship is not so sharply defined in regard to age, although since weight is to some extent a function of age there is some change there. Our experience corroborates Donaldson's tables in regard to the wide variations in weight in normal young adult rats, bred and kept under

conditions which are apparently the same. This table brings out the point that the absolute weight increases gradually with body weight.

These clearly defined results led to rearranging the data for all stages of the cycle according to weight. On the basis of the figures given in Table IV it was decided that all rats under 160 g. would form one group, those between 160–180 g. a second group and those over 180 g. a third group. The results of this rearrangement are shown in Table V. It is

TABLE V. Weight of the adrenals at various phases of œstrus grouped by weight.

	Body weight								
	Under 160 g.			160–180 g.			Over 180 g.		
Stage of cycle	No.	mg.	mg./kg.	No.	mg.	mg./kg.	No.	mg.	mg./kg.
Pro-œstrus	5	39·6±4·0	286±23	0	—	—	1	37·7	208
Œstrus	14	42·5±3·4	296±27	13	43·9±0·3	265±15	13	49·2±4·3	237±16
First day after œstrus	7	33·0±2·4	240±30	4	43·0±0·5	253±25	0	—	—
Second day after œstrus	19	36·0±3·2	249±23	8	41·4±0·4	244±26	6	42·0±2·4	212±6
Third day after œstrus	7	31·0±3·1	212±18	5	40·2±0·7	235±08	1	41·9	215
Day before expected œstrus	11	37·8±2·8	278±20	2	50·0±4·0	295±21	0	—	—

obvious that the greatest difference between the figures for both actual and relative adrenal weights for œstrus and dicœstrus is found in the lighter rats. The adrenal weight at œstrus shows a much greater variation than that in dicestrus, although in both cases it is greater in the young animals.

CORTEX-MEDULLA RATIO.

The relative volumes of the cortex and medulla of the ten rats in Series III are given in Table VI. The rats were killed at phases 2 and 5

TABLE VI. Weight of adrenal cortex and medulla.

				Adrenals			
	Rat No.	Age (days)	Weight (g.)	mg.	mg./kg.	Cortex mg./kg.	Medulla mg./kg.
Œstrus	1081 op.	61	129	34·7	270·5	253·7	16·8
Dicestrus	1068 op.	64	149	36·6	245·5	227·3	18·2
Œstrus	1061 c.	61	127	40·0	315·0	296·7	18·3
Dicestrus	1076 c.	62	142	35·8	252·1	235·1	17·0
Œstrus	1073 op.	62	170	44·4	261·5	244·5	17·0
Dicestrus	1079 op.	63	132	31·2	236·3	217·4	18·9
Œstrus	1058 c.	63	150	41·5	276·8	260·7	16·1
Dicestrus	1050 c.	64	149	32·5	218·2	199·6	18·6
Œstrus	974	73	168	45·0	268·0	251·7	16·3
Dicestrus	973	72	161	38·9	242·0	225·4	16·6

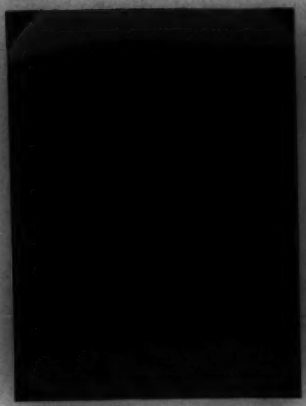
respectively. The mean percentage of medulla was found to be 6·08 ± 0·17 for œstrus and 7·51 ± 0·45 for dioestrus. The mean relative weight of the cortex as calculated from this percentage is 261·5 ± 12·2 mg./kg. for œstrus and 221·0 ± 8·3 for dioestrus. The mean relative weight of the medulla is 16·9 ± 0·5 mg./kg. for œstrus and 17·9 ± 0·6 for dioestrus. It will be noted that the difference in cortex weight for œstrus and dioestrus is significant, while that for the medulla is not, in view of the probable error. This means that the change in weight is due entirely to cortex.

Histological changes.

A comparison of the sections taken from rats killed at œstrus and at 60 hours after œstrus, under the standard conditions given above, reveals no change in the medulla as seen in the hæmatoxylin and eosin or fat stains. There is a change in the cortex (Fig. 2). In dioestrus the adrenal has a wide and clearly defined glomerular zone, outlined by a line of condensed nuclei from the fasciculatis. The cells in the outer portion of the fasciculatis are somewhat larger than those in the inner portion, but they contain few vacuoles. The cytoplasm is decreased in the inner zone and it merges without any sharp demarcation into the zone of cells bordering the medulla. Near the medulla and often lying adjacent to it are small groups of cells with an eosinophilic cytoplasm which is more abundant than that in most of the cells of the inner third of the cortex.

In the adrenal at œstrus the glomerular zone is narrower and less clearly demarcated from the fasciculatis. The cells of the outer half or two-thirds of the zona fasciculatis are much enlarged, pale and vacuolated. The margin between this area and the more compact cells of the inner third of the cortex is irregular and not sharply defined. The eosinophilic cells near the medulla are enlarged, contain vacuoles and resemble the cells of the outer portion of fasciculatis. Mitoses are extremely rare in the adrenal during both œstrus and dioestrus.

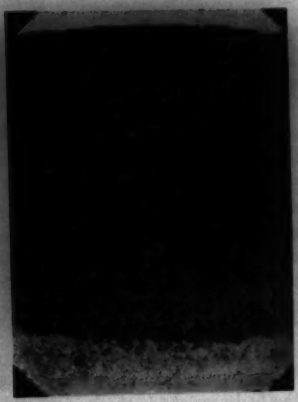
Scharlach R stains on frozen sections of the adrenal at dioestrus show an abundant deposit of large droplets of lipoid in the cortex which take the stain deeply. The margin of concentrated cells between the glomerular and fascicular zones and the more compact cells of the inner third of the latter do not take the stain. The nests of cells near the medulla do take it. At œstrus a few cells of the outer fourth of the medulla and some of the cells next the medulla contain large droplets which take the stain, but most of the cortex stains a diffuse pale red. Nile blue stains most of the cortical cells blue, although there is a little red-stained



1. Diœstrus. H. and E.

2. Œstrus. H. and E.

3. Diœstrus. Scharlach R.



4. Œstrus. Scharlach R.

Fig. 2.

material in the inner portion of the zona fascicularis. The blue is somewhat more abundant at œstrus, although the difference is not as striking as in the Scharlach R stains.

The adrenals in animals having irregular cycles or no cycles.

The data given in Table I show that the adrenal weight is quite low in animals having unusually long or irregular cycles. This led us to watch for animals which had had no cycles for some weeks preceding autopsy. Most of these animals were infected and therefore unsuitable for comparison, but four were found which were uninfected, and the adrenals in these animals were found to be quite small, and to have

Table VII. Rats that had no cycles for several weeks preceding autopsy. Ovaries without corpora lutea or large follicles.

Rat	Age (days)	Weight (g.)	Adrenals	
			mg.	mg./kg.
43–85	246	165	23	139
45–05	239	159	31	198
46–07	239	196	29	148
46–10	239	187	34	182

approximately the same relative weight as the adrenals of our spayed animals (data unpublished) (Table VII). Whether the lack of ovarian activity or the small adrenal is primary is, of course, still unknown.

Discussion.

The evidence presented points to a definite change in the physiology of the adrenal cortex associated with œstrus. Whether this is an increase or decrease of activity is still a problem. It also remains to be proven that this change signifies a specific causal relationship.

Conclusions.

1. The absolute and relative weights of the adrenal glands of the virgin female rat are greater at œstrus than at diœstrus, providing that the body weight is the same.

2. Rats having had irregular cycles or cycles of 6 days or over are found to have a lesser absolute and relative mean adrenal weight both at œstrus and diœstrus than those with regular cycles.

3. If the relative adrenal weights of the rats killed at œstrus are grouped according to the body weight of the rats, it is found that the absolute weight of both adrenal glands increases with body weight, while the relative weight decreases. The correlation is less close when the grouping is by age.

4. The relative adrenal weight of the rat in diœstrus is a fairly constant figure, regardless of the regularity of the cycle or the weight of the rat and is 0·20–0·23 g. per kg. body weight. The absolute weight is variable.

5. The difference between the mean relative weight of the adrenal gland at œstrus and diœstrus is therefore greater in lighter animals. In our series of 63 animals weighing less than 160 g. and having had regular cycles the mean relative weight at œstrus was 296 ± 27 mg./kg., and at diœstrus 212 ± 18. The difference in relative weight is about 25 p.c. of the figure for œstrus.

6. The difference between the mean absolute weight of the adrenal gland is also greater in lighter animals. The figures for the rats weighing less than 160 g. are: œstrus, 42 ± 3·4 mg.; diœstrus, 31 ± 3·1. This represents a difference of about 25 p.c. of the weight at œstrus, and of 11 mg. between the two means. In the rats weighing 160–180 g. the difference is 4 mg. or about 9 p.c., and in those weighing over 180 g., 7 mg. or about 14 p.c. The mean difference in actual weight is therefore in the same order of magnitude in these series, although the number of rats in the last two groups is not great enough for the actual figures to be final.

7. The increase in weight is found to be due entirely to increase in the size of the cortex. The cells of the zona fascicularis and of the cell nests near the medulla are enlarged during œstrus and contain an increase in lipoid which appears pale pink when stained with Scharlach R.

8. Since the weight and histological appearance of the adrenal of the unmated female rat is so variable it is suggested that, in experiments in which the sex of the animal is a matter of indifference and the weight or appearance of the gland is a criterion, male animals should be used.

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RETROGRADE POLARIZATION, A THEORY OF SYSTEMATIC ERRORS IN MEASUREMENTS OF MUSCULAR CHRONAXIE THROUGH RINGER'S FLUID OR WITH LARGE ELECTRODES[1].

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In the conclusions of a recent paper [Lapicque, 1931*b*] I recalled that chronaxie "reveals a deep modality, an essential property of each particular organization of the living matter"; this assumes that chronaxie depends only on the specific properties of the excitable tissue and not on the way the electric current is applied to it.

This proposition is essential. The various applications of chronaxie have been derived from it. These deductions have been verified by experiment, and thus we might consider the principle verified. But it is worth while to discuss the fact itself, considering only experiments on chronaxie measurements; for it has been contested on this ground, particularly with regard to the size of the electrodes.

In 1926 I was obliged to leave the question open, but I believe I am able to solve it now.

The discussion is related to muscle only; those who oppose my views admit that for nerve everyone is in agreement; though, even in this case, there is an appearance of changes in chronaxie under the influence of some experimental conditions, *e.g.* the chronaxie seems to depend on the distance between the electrodes. It will be interesting to discuss this fact now as an introduction.

We shall first give the experimental facts without using such abstractions as rheobase and chronaxie. The liminal potential for long durations and the liminal duration for a potential twice as high as the preceding one, do not depend on the distance between the electrodes above 1 cm.; the first increases while the second decreases when the distance between the electrodes is decreased below 1 cm.

[1] Translated from the French by Andrée Monnier-Dumont.

In order to deal with rheobase and chronaxie, the electric current must develop freely its physicochemical action in the tissue, the efficiency of this process on the tissue for stimulation being stopped at a given time by an effect depending on the tissue itself. It is this particular effect which, by its precocity or its delay, characterizes the tissue chronologically, and chronaxie must represent this very characteristic. If, for any reasons, another electrical disturbance hampers the action of the current on the tissue, before the stimulation process is stopped by the normal antagonistic process, we shall evidently miss the information we are looking for in the electric stimulation. For example, if the reverse polarizations which are developed at each electrode come in contact and neutralize each other, the limitation of the effect of the current would depend on something else than the tissue itself, and consequently the chronaxie measured is a false one.

This happens in nerve when the electrodes are only a few millimetres distant. Diffusion, as meant by Nernst (diffusion of a salt in water), would be much too slow to cause each polar perturbation to spread till it came in contact with the other. But the nerve (core conductor) contains in its axis a more conducting central part separated from a less conducting sheath by a polarizable surface. As soon as polarization starts under one electrode it spreads lengthwise, not by the effect of diffusion, but by the action of the current itself which, when opposed by the counter electromotive force that is just created, goes farther and farther to untouched surfaces. This phenomenon, known for almost a century, is the physical electrotonus. Its propagation is comparatively very rapid. From all we know, there is no difficulty in admitting that through this mechanism a notable transmission of positive ions might take place in a thousandth of a second through a centimetre, from the anode to the cathode; that is to say, some neutralization of the cathodic polarization. And this accounts perfectly for the so-called variations of rheobase and of chronaxie according to the variations in the distance of the electrodes.

But such a denomination is evidently inaccurate. There is only one chronaxie: that obtained with a sufficient distance between the electrodes to give the maximum value, which, above this distance, is independent of this experimental condition. This being the case, the value is conditioned only by the properties of the nerve itself and is the chronologic picture of these properties. On the contrary, when the electrodes are nearer to each other, the liminal duration observed is conditioned by a purely physical phenomenon and thus is not a chronaxie. We must not

say "chronaxie decreases with the distance between the electrodes," but "below a certain distance we are dealing, not with a true chronaxie, but with a pseudo-chronaxie deprived of any physiological significance."

The influence of the size of the electrodes on muscular chronaxie measurements offers a problem just the reverse of this one, or better, symmetrical with this one. Let us approach this question as it was presented by Davis [1923]. He says:

> The various workers have been in good agreement as to motor nerve....For striated muscle, however, Lapicque and his school find a chronaxie essentially the same as that of its motor nerve, while Lucas found it approximately ten to twenty times as long. The techniques of the two differ in that Lapicque stimulates the muscle with a small silver electrode, while Lucas used...his fluid electrodes. More recently, Jinnaka and Azuma have applied the pore electrodes...to the study of this problem, and have found values in general agreement with Lapicque...the different values are characteristic of the different techniques. The important variable is the size of the electrode. A small electrode, such as pores from 3 up to 75 μ in diameter, or a metal wire, gives short chronaxies, from 0·0002 sec. to 0·0005 sec., while fluid electrodes of the Lucas type give chronaxies up to 0·02 sec. By varying the size of the effective electrode, nearly the whole range of the intermediate values can be covered.

He concludes as follows:

> The pore electrodes resemble a little more closely, in point of size, at any rate, the physiological method of stimulation, *i.e.* by the nerve...but until the laws governing the wide variations can be worked out, it does not seem possible to speak of any single chronaxie as characteristic of a given muscle.

One would suppose that this note, without any numerical result and qualified by the author himself as "preliminary report," would have been followed by a more precise paper. To my knowledge, Davis has not published such a further paper. But a year later, Watts [1924] published a report, evidently a continuation of the work, using even Davis's own electrodes. These were, on the one hand, two Pratt electrodes, *A* with a pore of 3·5 μ in diameter, the other *B* with an elliptic pore of 73 × 44 μ; and on the other hand, *C*, a fluid electrode of Keith Lucas, with a slot of 5 × 1 mm.

These electrodes being applied on the pelvic end (nerveless) of a frog's sartorius, the following values were obtained as chronaxies, "representative values commonly obtained":

Electrode	Size in mm.	Chronaxie in σ
A	0·0039	0·15
B	0·073 × 0·044	0·55
C	5 × 1	2·7

Another Lucas's electrode with a slot 3·5 times as great—7·5 × 2·5 mm.—gave the same values as *C*; Watts takes no more account of this one, and from the three preceding ones, he concludes that Davis's demonstration of chronaxie as dependent on the size of the pore is confirmed.

At a first glance these figures may be impressive. But, if we consider the constitution of Pratt's and Keith Lucas's [1906] electrodes, of which Watts does not speak, we readily see that the conditions are not homologous.

Pratt's electrodes are made of thin glass tubes which are held perpendicularly to the muscle; the section in contact with the muscle is composed of a comparatively thick wall and of a microscopic hole (pore). The tube is filled with Ringer solution, through which the current is led. This is the cathode. In Watts's experiments the whole preparation, *i.e.* the whole frog with its muscle uncovered and left *in situ*, is immersed in physiological solution, the anode being dipped in this solution.

In Keith Lucas's fluid electrode the muscle is suspended inside a tube with a hole (slot) across its bottom. A more or less long part of the muscle goes through that hole; the part left above is maintained in physiological solution by atmospheric pressure, the top of the tube being closed by a cork, and the electric (negative) current being introduced through a lateral bifurcation. At some distance below this electrode, the inferior part of the muscle touches another physiological solution which constitutes the anode. Consequently, with Pratt's electrodes the current comes in contact with the muscle crosswise at a definite and limited surface. With Keith Lucas's electrodes the current goes lengthwise all around the muscle; near the hole the lines of current converge toward the muscle and penetrate all around it; the surface of penetration is not the surface of the hole, but the product of the muscle circumference by an undetermined length. Thus the two kinds of electrodes are completely different, and it is not justifiable to make a series in which the dimensions of the holes of one of them are compared with those of the other. The two cases must be studied separately.

The glass tubes applied at right angles to the muscle give a definite dimension for the electrodes; with these tubes we shall be able to determine if the dimension of electrodes has any influence on chronaxie.

In all my experiments, until 1930, I had never observed such an influence, but I always used relatively small electrodes, either thin silver wire or small brushes; now, I undertook to find out systematically if

there is any such influence. I found it practical to use my non-polarizable electrodes made of red glass and modified as follows: the end of the tube with the hole has been drawn and curved; by means of a short rubber tube, I could add interchangeable endings made of drawn glass; the free end of these endings is a cross-section, ground smooth on a very fine emery grinder. The dimension of the hole (as well as the thickness of the wall) is measured under a microscope of low magnification (× 30), with an ocular micrometer previously scaled to an objective micrometer. I prepared a series of such endings with different diameters, varying from 0·08 to 2·5 mm.; the thickness of the walls varied independently, from 0·3 to 1·5 mm. For my experiments I used the sartorius muscle of frogs, generally *R. fusca*, in which the dissection is easier. After the dissection, which must be done very carefully to avoid any injury to the muscle, the preparation is put in physiological solution to rest for about half an hour. Then it is fixed on the bottom of a small trough by two vegetable thorns which are pinned, not through the muscle itself, but in fragments of adjoining muscles left attached to it at each end for that purpose. The electrode described above, manipulated with a screw providing a fine adjustment, is placed at a right angle in the middle of the pelvic end, gently pressed against the muscle surface. The trough is filled with Ringer solution and a silver anode is dipped in it. Chronaxie is measured by means of a chronaximeter functioning by the break of a short circuit, giving in that way measurements within $0{\cdot}1\sigma$.

Here are the details of the first experiment, which gave already some significant results.

October 26. *R. fusca*; sartorius. Capillary cathode on the middle of the pelvic end. The anode is dipped in the solution, 1 cm. away from the distal end of the muscle.

Electrode *A*: diameter of the hole 0·12 mm., thickness of the wall 1·3 mm.; liminal voltage for 30σ (rheobase) 4·5; with a voltage of 9·0, the threshold is reached when the duration is reduced to $0{\cdot}4\sigma$.

Electrode *F*: hole 0·66; wall 0·46—rheobase 0·95; for a voltage of 1·9, liminal duration 0·4.

Electrode *B*: hole 0·15; wall 0·52—rheobase 2·2; for a voltage of 4·4, liminal duration 0·4.

The anode is changed to the pelvic side 1 cm. away from the end of the muscle.

Electrode *B*: rheobase 5·0; for a voltage of 10, liminal duration 0·3.

Electrode *A*: rheobase 4·0; for a voltage of 8, liminal duration 0·3.

The capillary electrode is replaced by a silver wire as in the experiments of my preceding papers [Lapicque, 1931]: rheobase 1·9; for a voltage of 3·8, liminal duration 0·7.

The results, within the limits of experimental variation with capillary electrodes, are, as to their liminal duration when the potential is double

that of the rheobase, independent of the instrumental conditions[1]. Thus they correspond to the definition of chronaxie. On the other hand, the value obtained with the silver wire, being about double, shows a slight extra phenomenon, in a way which we shall explain further; we must call this figure a pseudo-chronaxie.

The thickness of wall (the minimum being 1 mm.) has no effect. This may seem surprising when one remembers that chronaxie decreases when the electrodes are nearer to each other. Indeed, here the anode is diffuse around the cathode, whose conductivity is greater than that of the muscle, so that the distance between the point of entrance and of exit of the current (that is to say between the efficient electrodes) varies with the thickness of the wall. We have recalled how in nerve this condition leads to some important consequences. But there is no reason, *a priori*, to find the same physicochemical mechanism in muscle as that based on the existence of a more conducting core. In fact, I do not know any experiment made on muscle on the influence of the distance between the electrodes on chronaxie. It seemed useful, therefore, to take up this point.

We[2] operated on sartorius muscles either immersed in Ringer, or in the air after a long rest in the solution. (The muscle is then wiped before the experiment.) We took two of our capillary electrodes or only one and a silver wire, both being placed on the nerveless region of the muscle, lengthwise, in order to make the current flow in more or less long portions of fibres and not jump from one fibre to another. When we increased or decreased the distance between the electrodes we observed, in some cases, irregular variations which had no relation to the distance between the electrodes. As a rule the result is the same, within the range of experimental error, whether the electrodes are at 6 or 2 mm. apart. If we put them very far apart, 25 or 30 mm., *i.e.* almost across the whole length of the muscle, we still obtain the same figure as for any possible distance within the pelvic end, but we do not then know whether or not we have provoked a nervous stimulation; at any rate, if the electrodes themselves are not able to introduce an α effect, according to a mechanism which we shall explain, the distance between them has no effect on chronaxie.

[1] The variation from 0·4 to 0·3 when the position of the anode is changed is not significant, as the determinations are accurate only to within a tenth of a σ. However, the temperature having risen slightly during the course of the experiment, there might be actually a slight decrease in the chronaxie, at any rate, not correlated with the direction of the current.

[2] Every experiment has been performed with Madame Lapicque.

Thus it is no longer surprising that the thickness of the wall showed no influence.

Let us go back now to our main question: that is the influence of the diameter of the conducting electrode in its insulating sheath, in other words, the inside diameter of our capillary endings.

All the following experiments have confirmed the first one, and they emphasize a general result which we can formulate in this way. From the smallest diameter used, one-tenth of a millimetre, to five or six-tenths of a millimetre, the size of the electrodes has no influence whatsoever.

But above six or seven-tenths of a millimetre the minimal duration for a potential twice that of the rheobase increases regularly with the diameter of the ending. This increase, already conspicuous for a diameter of 1 mm., reaches several σ for a diameter of 2·5 mm.

Here are the figures of a few experiments given in the order they were made; in each couple of figures, the first one represents the diameter of the hole in mm.; the second, in heavy type, the duration found in thousandths of a second.

October 27: 0·36, **0·4**; 0·15, **0·3**; 0·66, **0·3**; 1·5, **1·0**; 0·66, **0·4**; 2·0, **1·5**; 1·0, **0·6**; 2·5, **3·0**.

October 28: 0·66, **0·5**; 0·24, **0·3**; 0·15, **0·3**; 1·5, **1·3**; 2·5, **2·8**; 0·45, **0·4**; 1·0, **0·7**.

November 3: 0·36, **0·5**; 1·0, **0·6**; 0·15, **0·55**; 2·5, **2·5**; 1·0, **0·6**; 0·80, **0·55**; 0·15, **0·50**; 0·08, **0·45**.

November 14: 0·15, **0·3**; 0·30, **0·3**; 0·40, **0·3**; 0·90, **0·5**; 2·5, **1·2**.

These values plotted graphically (abscissæ: diameters of the electrode; ordinates: liminal duration for twice the rheobase) outline systematic curves, each one tending, when starting from large sizes, distinctly toward a constant value which is practically reached between 1 and 0·5 mm. For smaller sizes the variations obtained in our measurements are within the range of experimental error.

We have not in fact used any diameter smaller than about one-tenth of a millimetre. Watts used a much smaller one, of the order of a thousandth of a millimetre, but the values he obtained are hardly smaller than ours; the author gives 0·15σ as a representative figure, but his experimental figures range from 0·12 to 0·35σ. Furthermore, Jinnaka and Azuma [1922] who were the first to make measurements with Pratt's electrodes, found, with a diameter of 10μ, chronaxies of 0·2 to 0·3σ for the sartorius. Thus, from the experimental point of view, it is not likely

that the curve, after it has reached a constant value for diameters of a few tenths of a millimetre, should present a new decrease for much smaller diameters.

Outside the range of constant values, the important fact is an ascending slope of the curve toward large diameters, but, as we noticed that for 2·5 mm. we obtained figures around 3σ, it is worth while to recall values obtained with the bath constituting a "fluid cathode" when the length of the muscle exposed to the current is progressively reduced. The pseudo-chronaxie decreases from 20σ for half of the muscle

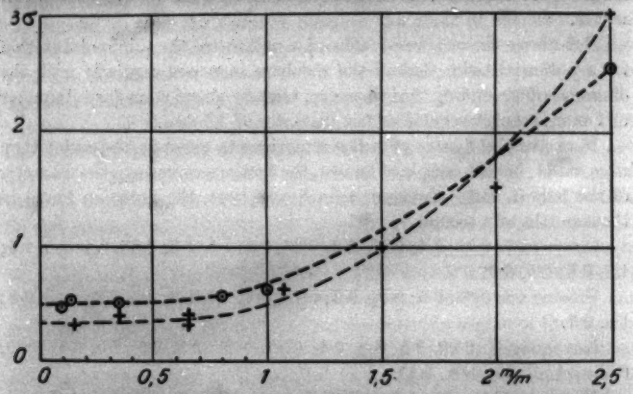

Fig. 1. Two experiments, each one demonstrating the pseudo-chronaxie (as ordinate), raising above the chronaxie (on the left side) according to the diameter of electrode (as abscissa).

to 3σ for 2–3 mm. [Lapicque, 1931 *b*, p. 220]. In other words, the capillary electrode on one side and the solution as used by Rushton on the other give the same chronological characteristic for stimulation when the same length of muscle undergoes the action of the current through the Ringer. The two series of measurements join each other within the limits of experimental error; Fig. 2 illustrates this point. Thus it may be quite possible that one and the same phenomenon is involved for the ascending part of the curve with one or the other apparatus. I previously called this phenomenon the α effect. Every chronological characteristic located on the ascending branch of the curve or on the part which joins it with the horizontal branch, must be, for one and the

same reason, called a pseudo-chronaxie. The horizontal branch is the only location of true chronaxies, that is to say, independent of instrumental conditions, but determined by the muscle's own properties.

There is not only an abstracted, a theoretical limit when the size of the electrodes tends toward 0, as Davis thought. There is, practically, a notable range of dimensions where this value is not a function of the calibre of the electrodes. This circumstance is convenient; from the practical point of view, we do not need to take into consideration the calibre, if we keep it below a given dimension, and that is easy to estimate

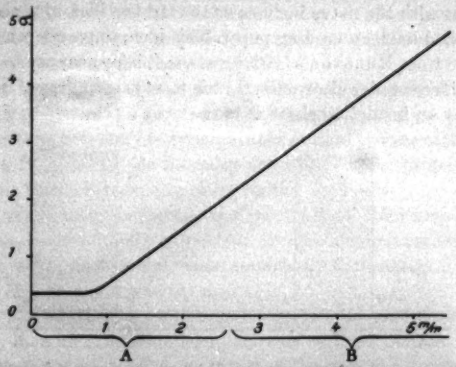

Fig. 2. Schematic graph, to show how experiments with electrodes of varying sizes (in *A*) join experiments in bath of varying lengths (in *B*). Chronaxie or pseudo-chronaxie as ordinate, length of muscle submitted to electric current as abscissa.

directly as we have always done in our previous experiments in a purely empirical way.

We can now see the very symmetrical correspondence between muscular stimulation and nervous stimulation; for nerve, the electrodes must be kept apart at a distance at least equal to a certain minimum, otherwise the chronaxie measurement is falsified by the reciprocal action of electrotonic polarizations. For muscle, the cathode must remain, as to its size, below a certain maximum dimension, otherwise the measure is falsified by an α effect. Though this effect had been left undetermined, our previous results [Lapicque, 1931 *b*] show again a great symmetry between the conditions for this effect on muscle and the conditions which

provoke an electrotonus on nerve; for nerve the current reaches the excitable part, the axone, through a less conducting sheath; for the α effect, the current reaches the excitable part, the muscle itself, through a sheath of Ringer more conducting than the muscle itself. We can conceive that these two opposite conditions, if combined, might more or less neutralize each other. This could explain how a nerve electrically stimulated through Ringer solution might present but a slight α effect [Lapicque, 1931 *b*, pp. 237 *et seq.*].

Now if we try to imagine what the α effect might be in itself, this comparison with the nerve inclines us toward the idea of a polarization, as I suggested in the preceding paper. This idea, unprecise when apparatus derived from Rushton's [1930] was used, becomes easy to determine if we consider tubular electrodes, that is to say, cylinders of Ringer surrounded by an insulating sheath.

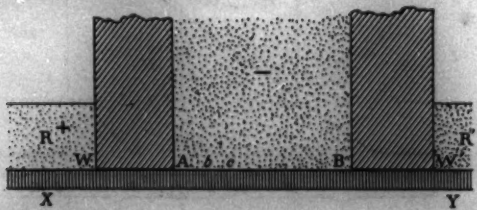

Fig. 3. Theoretical figure for the understanding of the retrograde polarization (see text).

Let us consider a muscle represented by a single fibre[1]; we shall use as a basis for our scale its crosswise dimensions. *AB* is the diameter of the Ringer solution which touches the fibre; *AW* and *BW'* the cross-sections of the wall on the same diameter; *R* and *R'* the Ringer solution outside the negative electrode. Suppose the anode is placed in this solution on the left of the figure (in *R*). When we create a potential difference in the circuit, the stimulating (*i.e.* negative) current must pass from *AB* to *R* through the muscle; of course, at first it will mostly pass in *A*, as in a vertical cylinder of Ringer the way to any point in *AB* offers the same resistance; but the next conductor constituted by the muscle offers a resistance proportional to its length, therefore weaker

[1] Concerning the muscle, the word fibre is not unequivocal; I mean Leydig's primary bundle, which I now call myon, that is to say the functional muscular element, just as a neuron is the functional nervous element [Lapicque, 1932].

from *A* than from any other point and becoming greater the farther we go from *W*. But the muscle surface is polarizable, which means that a counter-electromotive force is developed on each surface element, more or less rapidly, according to the intensity of the current going through this element. Thus *A* will be polarized most rapidly and then the current will be diverted toward *b*, where polarization will again increase and divert the current still farther. When the polarization thus reaches *B*, the current will be obliged at last to overcome directly the counter-electromotive force of polarization and to finish, in the muscle, the development of the process from which stimulation arises.

We see then that the minimal time during which we must keep the circuit closed for a given potential, in order to stimulate, is composed in the abstract of two distinct elements:

(1) the time necessary for polarization to develop itself along *AB*, time which of course is a function of this length;

(2) the time necessary to terminate the normal process of stimulation, which is a function of the intensity according to the properties of the tissue. This factor is normally given by the chronaxie.

With very small electrodes, the polarization difference between *A* and *B* can be practically neglected. We are dealing purely with the excitation time, and under those conditions the liminal duration for twice the rheobase is a true chronaxie.

When *AB* becomes longer, we have, for each intensity, first a polarization time, then an excitation time; the sum of these two time factors is, necessarily, for any intensity, greater than the pure and simple excitation time; this is particularly true when after the measure of the liminal intensity for long durations we look for the liminal duration with a double intensity. Whether the first intensity differs or not from the real rheobase, the duration obtained is different from the chronaxie; we readily see how right we are in calling it a pseudo-chronaxie; it is always greater and may become considerably greater if polarization takes a long time, and this happens when it has to cover a large surface. With an electrode of 2–3 mm., the pseudo-chronaxie is 2–3σ. We can conclude that here the polarization time already constitutes the greater part of this duration.

With Rushton's apparatus, where the whole muscle is dipped in Ringer, the current reaches it lengthwise through the solution; the physical reasoning is slightly different and we must take into consideration the difference of conductivity between the solution and the muscle.

But, in such a bath, the problem is not definite even if we localize the anode or the cathode in the bath by means of a metallic conductor as I did. I had supposed already [Lapicque, 1931 *b*, p. 242] that it was necessary to think of a polarization pushing back the current from spot to spot, but I did not know where to place the starting-point.

The following apparatus allows us to pass from one case to another and to determine the phenomenon which is common to both. Let us take a layer made of a given number of pieces of filter paper, 5 or 6 mm. wide and about 10 cm. long; let us soak it in Ringer and lay it horizontally between two vertical blades made of chlorinated silver; we then pass an electric current through it lengthwise. A carefully dissected sartorius, having been immersed in Ringer for more than half an hour, is placed flat and lengthwise on this pad. We can readily avoid the singular points constituted by the ends of the muscle in placing these on a thin glass slide (microscopic cover slide). The whole preparation is placed in a rectangular trough, made of parallel glass plates and covered with the same in order to avoid drying, without hindering observation of the muscle.

In this position we can easily stimulate the muscle with potential differences of a few volts; the curves obtained in this way are a mixture of α, γ, and even δ curves.

This is an example.

Exp. November 23. Sartorius of *R. fusca* placed as described above, with the cathode first on the distal side, *S*, then on the pelvic side, *P*.

	Liminal voltage	
Duration in σ	*S*	*P*
50	—	10·2
20	8·3	10·6
10	—	12·5
5	8·3	14·2
3	8·3	15·3
2	8·3	—
1	9·0	15·5
0·5	10·6	17·2
0·4	—	18·5
0·3	13	—
0·2	16	—

It is obvious that *S* is a γ curve; *P* starts in an α curve interrupted around 4σ by a γ curve. In other words we get exactly the same results as in a bath[1].

[1] This experiment is particularly similar to one published and illustrated with a graph in a preceding paper [Lapicque, 1931 *a*, p. 203].

But we can follow more clearly the nature of the response and particularly the change at the time when the curve is changed into a γ one, that is to say when the direct muscular stimulation becomes a stimulation through the nerve. Besides, we can observe the localization at the very beginning of the muscular response; we shall consider this point again further on. With this apparatus, the notion of parallelism without distortion of the lines of current, which was the original desideratum of Rushton's work, proves to be entirely delusive. This parallelism exists only in the pad, when considered alone, but it cannot subsist when the current goes through the muscle and stimulates it; one can see that it must deviate, and divide, at first according to the relative conductivity of the two superposed conductors, muscle and pad; then immediately after it must undergo some considerable distortion owing to the polarization of the muscular surface[1]. But here, no more than with the bath, there is no definite starting-point for polarization. The following procedure will give us one.

Let us cut into equal parts the pad soaked with Ringer and put them about 1 cm. apart; then fill this space with an insulating block (paraffin) of the same thickness. The muscle is then replaced on the pad as previously, but now it constitutes a bridge between two conducting parts and the whole current must pass through it. Let us place a sartorius in this position; its middle part on the paraffin, its extreme pelvic end on a slide and the part in between, 6 or 8 mm. of muscle, on the wet pad. The cathode being under the pelvic end, the curve obtained is a mixed α and γ curve.

Example. Exp. of January 4. 7 mm. between the slide and the paraffin.

Duration	Liminal voltage
100σ	8·2
50	8·8
40	9·6
30	10·6
20	11·9
10	12·7

The response of the muscle to this last stimulation is a strong contraction, even for the threshold current, which means that we have begun to stimulate the nerve; and if we continued from there toward

[1] I started with A. M. Monnier to study such distortions with the cathode ray oscillograph. On preliminary trials we found that the current going from a given point of the pad to one point of the muscle shows, in terms of time, a very complex shape, so complex that they could not be analysed in a rapid study.

short durations we could get a γ curve. The figures for longer durations belong conspicuously to an α curve which shows, by extrapolation, a pseudo-chronaxie of 8 or 10σ; this is the same length relation as with the other apparatus. We must be dealing with the same mechanism.

We can reason in the following way: the negative current will penetrate the muscle on the whole length bg, but the bulk of soaked filter paper, F, being more conducting than the superposed muscle, the intensity of the current will be the greatest in b and the smallest in g; so that b will be polarized quicker than any other point. This is the same case as the one described above, when the current arrives at a right angle in contact with the muscle, through the electrodes. But now the part played by the conductivity of the medium outside the muscle, as it had been observed in the bath, is obvious.

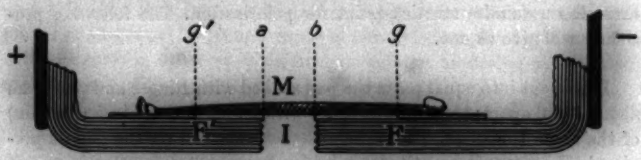

Fig. 4. Experiment upon an interrupted pad (see text).

When the retrograde polarization has covered the whole length bg, the current, as we have said, will be at last obliged to complete the stimulation process. But where will stimulation start? It would be rather hard to foretell it by *a priori* reasoning. If we did not take into consideration the interfacial polarization we should evidently think of it as occurring in b, at the end of the more conducting electrode, where the intensity of the current is the highest; but there the intensity will decrease gradually while it will increase in g; the question becomes very complex; and even if we knew the exact shape of these varying currents, it would still be difficult to foretell which one will reach the threshold first.

Observation answers very clearly: at least for the conditions of resistance of my experiments, stimulation starts in g and even 1 mm. back, upon the glass slide.

If we did not bear in mind the idea of a retrograde polarization, this result, I think, would seem paradoxical. This paradoxical character becomes still more striking if instead of the soaked paper we use a metallic electrode.

Let us consider a silver blade pp', with the edge p well rounded; it is held by an insulating block *I*. We put, on this blade, the pelvic half of a sartorius, the hanging distal end of which is dipped in a Ringer bath *R*; a glass slide g insulates, as previously, the pelvic end. The silver blade constitutes the cathode, and the bath the anode. We get well-characterized α curves for durations around a hundredth of a second; for durations of thousandths of a second, where consequently the potential must be high to reach the threshold, the results are very irregular, and furthermore, they leave some permanent changes in excitability.

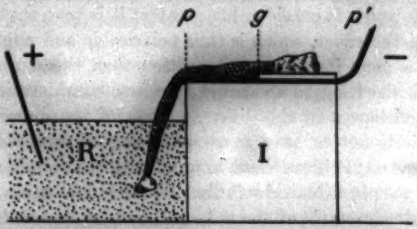

Fig. 5. Experiment upon a large metallic cathode (see text).

But the series of measurements made only with long durations give a very definite and perfectly significant strength-duration relation. They give a pseudo-chronaxie related to the muscle length lying on the electrode which is an exact agreement with the preceding results.

Example. Exp. January 7. 7 mm. between p and g. Cathode connected in p'.

Duration	Liminal voltage	
100σ	4·0	3·0
50	4·3	3·0
30	4·5	3·4
20	5·0	4·0
10	6·8	5·4

The two series of liminal potentials have been determined in the same order as given in the table; there were, between the two, a series of trials which did not give a consistent threshold with a duration of 5σ; these two series give very similar curves with a slightly extrapolated pseudo-chronaxie of about 8σ for each one.

When the direction of the current is reversed, we get a γ curve with a chronaxie of 0·4; the response is *d'emblée* large and characteristic of nervous stimulation. When p' is negative and the duration short, the

irregularity of response is accompanied by irregular thresholds; but in the series of determinations given in the table, that is to say purely in the range of the α effect, the first response, the real threshold, appears on the edge of the slide g; if we increase very gradually the intensity of the stimulus we can see the increase of the local response *pari passu*, and then for an intensity still slightly greater, it spreads all at once to the whole length of a few fibres.

In short, we get exactly the same phenomenon as with the preceding apparatus; and, what is still more remarkable, the same chronological characteristic for the same length of muscle, in spite of the difference of conductivity which is considerably greater; it is true that the blade is made of polished silver, which is thus polarizable and might have been the seat of a more important polarization than the muscle surface in contact with the Ringer; we should take into consideration the distribution of resistances in the different parts of the circuit. But these details, seeming not to have much physiological importance, are not worth much work; it is sufficient to note, with the metallic electrode as well as with the physiological solution: (1) the introduction of an α effect with a pseudo-chronaxie of about 1σ per mm. of muscle length undergoing the action of the current; (2) the origin of stimulation at the point of contact between the muscle and the electrode nearest to the origin of the stimulating current; that is to say the reverse of what a reasoning based only on ohmic resistances would give.

The case of the silver wire coming in contact with the muscle through the solution, and which at first approximation I considered as "stigmatic," is in fact a mixed case; the surface by which the electrode itself comes in touch with the muscle is evidently within the limits where the retrograde polarization is negligible; if the current reached the muscle only through this surface, we should get a correct chronaxie; but the bath, with its intermediate conductivity, diffuses above its end a portion of the current which is not negligible; thus our efficient electrode is wider than the wire; the value of the pseudo-chronaxie obtained with this procedure, $0{\cdot}7$–1σ, suggests an effective size of about 1 mm.

The same thing happens when the glass capillary electrode is not applied closely to the muscle surface; if its section makes a slight angle with it, or is slightly above, leaving a small space where the current can spread, the liminal duration for a current twice the rheobase appears increased, sometimes considerably. This can be easily understood and can be experimented upon directly; we have only to repeat the two measure-

ments (rheobase and chronaxie) at the same time as we change the height of the electrode by means of a micrometric screw; provided that the electrode obviously touches the muscle its pressure has no effect on chronaxie, but when it is raised above a certain level the smallest rise increases it notably, and it may become twice or three times larger. Evidently we are now dealing again with a pseudo-chronaxie similar to the one obtained with wide electrodes.

So that in order to have a correct measurement of a muscular chronaxie we must use either a metallic wire touching the muscle in the air[1], as we had commonly done until now, or a liquid electrode contained in an insulating sheath and whose diameter is less than $\frac{1}{4}$ mm.

Let us go back now to Keith Lucas's fluid electrodes. This is the way in which we can imagine these phenomena; the negative current penetrates first in the muscle at the level of the slot where it emerges from the Ringer, in the same way as at the end of the wet pad in contact with the paraffin; then a retrograde polarization starts and goes upward along the part of the muscle suspended above in the electrode; then, and then only, does stimulation take place; for the pure muscular stimulation this is true only when the slot is placed on the nerveless portion of the muscle.

[1] We proceeded in this way in most of our researches on the gastrocnemius; we considered the sartorius unsuitable on account of its variable increasing chronaxie and of its rapid loss of excitability. These reasons led Keith Lucas to devise his "fluid" electrodes in order to avoid desiccation and temperature changes which, he thought, were the causes of this variability.

But Dulière and Horton [1929] and Horton [1930] in a very elegant piece of research, whose practical importance seems to me very great, have shown that this muscle, and the others to a certain extent, are subject to a reversible loss of excitability, which takes place at ordinary temperature in a moist atmosphere without any desiccation and which returns when the muscle is thoroughly washed in Ringer; besides this alteration can be prevented if we previously wash the muscle in Ringer to take away a fraction of its potassium.

Hill, in his famous experiments on thermic phenomena of muscular contraction, had found it necessary, empirically, to keep the sartorius in a Ringer bath; Keith Lucas's "fluid" electrodes, though conceived with a different theoretical idea, met the same requirement; they ought to preserve the muscle excitability, but they introduced, in the way that we shall explain hereafter, the α effect; in which, at first, I thought I recognized the alterability mentioned above.

To-day, after Dulière's and Horton's work, we can use the sartorius without any hesitation for excitability researches. This muscle is particularly convenient for many physiological purposes on account of its anatomical conditions, and, as a matter of fact, it had been very often chosen for researches. It keeps perfectly in Ringer and, concerning chronaxie, gives correct values deprived of any α effect with capillary electrodes of an inside diameter of a few tenths of a millimetre.

This conception quantitatively accounts for the different results obtained by the authors: that is to say,

(1) The dimension of the slot has no influence whatsoever on the chronological characteristic of the intensity-duration relation. Indeed, Watts found no difference for two slots being in the ratio 1–3·9.

(2) The pseudo-chronaxie which measures this characteristic varies from 2 to 6σ. In our conception, this value must be a function of the length of the muscle undergoing the lengthwise action of the current; our experiments have found it to be approximately proportional to this length, about 1σ per millimetre. Here the length to be taken into account is the one of the muscle left above the slot. The authors did not indicate this length, as they did not suspect its interest. But both Keith Lucas and Watts, when taking care to place the slot on the nerveless portion of the sartorius, must have left only a few millimetres above the slot, and this agrees exactly with the values they obtained for the pseudo-chronaxie.

Summary.

Measurements of chronaxie have been made, on the pelvic part of the sartorius previously bathed in Ringer's fluid, with electrodes made of capillary glass tubes of varying diameters. Between 0·1 and 0·5 or 0·6 mm. the chronaxie remains fully invariable and equal to the one given by a silver wire taken as electrode on a muscle in air. The value so found, 0·3σ as a rule, is the true chronaxie. From 0·5 to 0·6 mm. up, chronaxie, or better pseudo-chronaxie, increases regularly with the size of the cathode, reaching a value as high as 2 or 3σ for a diameter of 2 or 3 mm.

It is recalled that experiments made following Rushton, *i.e.* when a muscle is stimulated through a bath of Ringer's fluid, give pseudo-chronaxies regularly varying with the length exposed to the current, viz. 2 or 3σ for 2 or 3 mm., and about 1σ more for each additional millimetre. Thus, both series of experiments join exactly.

Reasoning physically from the case of large capillary electrodes, one must admit a polarization of the muscle surface under the cathode, starting from the edge at the point nearest to the anode, and developing itself progressively as far as the most remote point of the cathode. So, the current crossing the muscle surface is diverted backward all along the diameter of the cathode. In order to reach the threshold, the circuit must remain closed a sufficient time to allow (1) this polarization to come to its end; (2) the proper excitation process to be achieved. This sum

appears in experiments as pseudo-chronaxie, evidently longer than the true chronaxie, and increasing *pari passu* with the length of muscle subject to the phenomenon.

Laying the muscle upon a pad soaked with Ringer's fluid and sending an electric current in parallel lines, we find the same α and γ curves as in a full bath. This still holds when we interrupt the pad with a block of insulating substance and bridge this electric gap with the muscle. Such a case appeals to the same physical reasoning as the case of a large capillary electrode, but involves as a necessary condition a greater conduction in the electrode than in the muscle.

Every condition and every phenomenon related to this retrograde polarization for the muscle is symmetrical to the physical electrotonus in the nerve, where, as classically and rightly admitted, the excitable substance (axon) is surrounded by a less conducting substance.

Post-scriptum versus W. A. H. Rushton.

Following the report [Rushton, 1930] which I discussed in my preceding papers [Lapicque, 1931 *a*, *b*] and am still discussing in the present one, Rushton has published several papers on the same subject, bringing forward, with much application and ingenuity, new and detailed evidence concerning the fundamental phenomenon which is the basis of the discussion, viz. the existence of two different strength-duration curves for the nerve-muscle complex when stimulated electrically through Ringer's solution. I have said already, and willingly repeat, how much I am indebted to Rushton for having made this fact unquestionable even in normal fresh muscle, and so for affording the opportunity of evaluating the rôle played by the electrolytic medium. But, I must confess, I can hardly find in all this secondary work any new light on the physiological problem concerning the interpretation of the α curve and consequently the question whether the neuromuscular isochronism is right or wrong.

In a first paper [Rushton, 1931] he shows that the phenomenon is present, generally speaking, in any one among the frog's striated muscles. Since I discarded, the moment I read his primary report [Rushton, 1930], my old tentative explanation of the Lucas's α curve, I never regarded such a generalization as in doubt and I even admitted it implicitly, considering at every theoretical standpoint, the *muscle*, or the *muscular substance*, without any restriction to a particular case. Though I have borne in mind a special condition for the Gastrocnemius, the customary object of my previous experiments; here the muscular fibres, admittedly able (theoretically) to give an α effect as well as those of any muscle, are geometrically ordered according to the well-known penniform structure of the whole muscle; thus they present only a small part of their length freely to the current flowing in the Ringer; such a condition ought to restrict the phenomenon. In fact, in this muscle, Rushton found but a very small difference between the two curves, so small that plotted in the same way as other muscles, it was not conspicuous at all, and appeared only with special contrivances [Rushton, 1931, p. 270]. [Incidentally, one may note that the change in latent period showed by Rushton [1931, p. 275], when stimulation passes from γ to α, agrees fully with my theory stated above, viz. retrograde polarization.]

In a second paper [Rushton, 1932 *a*] he shows that the muscular fibres giving the α curve are those responding to the nervous stimulation. I do not know that anybody

has ever ventured the contrary hypothesis, surely a very strange one. For my own part, I did not raise any question about this connection, in the whole course of the discussion I gave in two reports published in this *Journal*, 5 or 6 months before Rushton's quoted paper was printed, but, unfortunately, after it was written, as we learn from a footnote. I understand that Rushton would not throw in the waste-paper basket a very industrious work, in any case interesting from its technical ingenuity, but at the time of publication he could certainly have left out such a cutting conclusion as the following: "The α fibres being supplied by nerves and having a chronaxie of the order of hundred-times as long as nerve... the conclusion leads inevitably to the rejection either of Lapicque's theory of isochronism or of the block electrode method of obtaining the chronaxie."

I quite agree to the position of the dilemma, but the question of the legitimacy of the said block method was precisely raised by myself half a year before, with a sufficient emphasis, I supposed; I had given a series of arguments against this legitimacy, and I was anxious to know what criticism would meet these arguments; but Rushton's paper remains systematically ignorant of them, and, furthermore, assumes without reserve as well as without justification that it was dealing with an alpha substance or even, as we have seen in the sentence quoted above, alpha fibres, a new and aggravating expression, though, in the preceding paper [Rushton, 1931, p. 265], a footnote admitted that two modes of excitability are not a sufficient evidence for the existence of two substances.

In a third paper [Rushton, 1932 *b*] we get the discussion, but not yet in the depth of the problem, only on a theoretical consideration raised by myself merely as preliminary. We must now satisfy ourselves with this unimportant discussion, for the fourth paper [Rushton, 1932 *c*], the last at the moment, is devoted to the demonstration that the γ curve, as a rule in a Ringer's bath, when the field of action to the electric current is large, is related to the nerve; again a good piece of ingenuity and precision, but quite superfluous, the fact being not denied.

The fundamental question is to know if the slow chronological character of an α curve means a large chronaxie for the muscle on which it has been obtained. I said no, and I say no.

Rushton seems to believe that my criticism aims only at the wording, and in deference to my rights as author of the name chronaxie, he concedes this name "to be used in the sense which Lapicque wishes"..."in the case where the strength-duration curve is canonical or where it satisfies any new criteria which Lapicque may in future suggest" [Rushton, 1932 *b*, p. 439].

I am certainly not inimical against an ironical joke in a scientific discussion, but I think it cannot be substituted for real arguments. This canon, that Rushton endeavours to turn to ridicule, has been imagined with the aim of compelling Rushton himself to acknowledge a notable difference between the α curves and the time relation having given birth to the chronaxie; if so, the duration corresponding to twice the liminal strength for long durations, in an α curve, should evidently not be homologous with the chronaxie and could be named a false chronaxie. Rushton considers this reasoning as an arbitrary fancy of my own; furthermore, he lays stress on this fact, long ago recognized by myself, that with the very rapid nerves and for durations shorter than the chronaxie, the strength-duration curve diverges from the general law. I had formerly imputed this deviation (we are dealing with durations of a tenth of a sigma, and below) to the self-inductance in the stimulating circuit, maybe wrongly (still I was not guilty of a certain technical fault that Rushton attributes to my experiments in contradiction to the very text of my report); that is a question concerning the physiological theory of the stimulation, but neither the measurement of the chronaxie, nor the said canon, since the alleged error lies outside the part of the curve used in the computation. I have twice asked Rushton by letter to let me know the figures he obtained between rheobase and chronaxie, the only

figures bearing on the case; he did not answer; thus I suppose his figures have been canonical, as well as those of any author, except perhaps very close to the chronaxie itself, where there may already exist a deviation of but a few units p.c., *i.e.* of the order of the experimental approximation. That is by no means comparable with the deviation offered by the α curves, chiefly on the side of long durations, and giving frequently incertitudes of a 100 p.c. in the position of their chronaxie, or better pseudo-chronaxie [Lapicque, 1931 *a*].

But whatever the criticism against it, the canon itself matters now very little. Its value was only previous and temporary, indicating that another quantitative relation could mean another quality of phenomenon. I have tried to find this other phenomenon, and in due course, I hope I have succeeded in tracing it. "Until the laws governing the wide variations (according to the size of electrodes) can be worked out, it does not seem possible to speak of any single chronaxie as characteristic of a given muscle" said Davis nine years ago, in a sentence quoted in the above paper and recalled by Rushton. Perhaps, the above report gives such laws, and shows when and how a neatly defined chronaxie exists for a given muscle, being equal to the chronaxie of the corresponding nerve, and when and how a parasitic phenomenon falsifies the strength-duration relation, leading to senseless chronological values. The time constant of an α curve, theoretically suspected at first because uncanonical, is now experimentally convicted as a false chronaxie, without any physiological meaning.

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STUDIES CONCERNING THE ALIMENTARY ABSORPTION OF WATER AND TISSUE HYDRATION IN RELATION TO DIURESIS.

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Part III. The influence of posterior pituitary hormone on the absorption and distribution of water.

Introduction.

It is known that the diuresis which normally follows a large dose of water is inhibited by pituitrin and likewise the polyuria of diabetes insipidus is usually reduced. It was shown by Verney [1926] that the abundant hypotonic urine formed by isolated kidneys when perfused by a simple heart-lung preparation is both reduced in amount and increased in concentration by pituitrin or by the inclusion in the blood circuit of a head, provided the pituitary gland of that head remains intact. Although the polyuria of isolated kidneys is not a water diuresis, since no additional water has been given, the inhibition of their activity by pituitrin is an indication that the hormone of the pituitary body has a direct renal action and suggests [Verney, 1929] that this inhibition of water diuresis is primarily of renal origin. This is further supported by the observation of the same author that in unanæsthetized dogs with denervation of one kidney the urine flow from the ureters is normally equal and the kidneys respond equally to water diuresis and are equally inhibited by pituitrin. On the other hand, except by some extrarenal action, it is difficult to explain the results of Miura [1925], and Buschke [1928], who observe increases in the blood chlorides when pituitrin is given to nephrectomized rabbits.

Our object is to examine the effect of this hormone upon the absorption and distribution of water administered by stomach tube to intact unanæsthetized animals.

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Firstly, we have administered water to rats and compared the normal absorption curve with the absorption curve after pituitary extracts by killing sets of rats at varying times after water was given and determining the amounts of water remaining in the alimentary canals. Secondly, in rabbits we have obtained for analysis samples of muscle, skin and blood during the course of the experiments by the procedures which have been shown in the first paper of this series to leave the diuresis uninfluenced and afterwards we have made post-mortem examinations.

A. The absorption of water from the alimentary canal of rats under the influence of pituitary hormone.

Experimental procedure.

The rates of water absorption of rats which have received about 1 unit of pitressin per 100 g. rat subcutaneously 30 min. before the administration of 5 p.c. of their body weight of warm water have been compared with the absorption rates of rats receiving only the dose of water. The method of study is precisely as described in the first paper of this series, Part I, Section B.

Results.

Table I gives the weight of gut and contents at varying times after administering 5 p.c. of the body weight of water. The left-hand columns are control experiments, the right-hand columns give the results on animals under the influence of pituitrin. It will be evident from Table I and Fig. 1 that the pituitary hormone has no significant influence on the water absorption rate in rats.

Table I.

Time after giving water (min.)	Rats without pitressin			Rats with pitressin		
	No. of rats	Weight of gut as p.c. of body weight	Average	No. of rats	Weight of gut as p.c. of body weight	Average
15	3	8·9, 8·5, 9·2	8·9	4	10·4, 8·3, 7·1, 8·9	8·7
30	4	9·6, 6·5, 4·8, 6·9	7·0	4	7·2, 6·7, 6·4, 6·8	6·8
45	6	6·9, 6·2, 6·4, 6·1, 6·2, 5·7	6·2	6	5·2, 7·1, 6·2, 7·6, 6·6, 6·4	6·7
75	7	4·6, 10·3, 6·4, 7·9, 5·9, 5·5, 4·9	6·5	7	4·8, 8·4, 6·6, 8·9, 10·9, 4·7, 5·8	6·9
120	6	6·8, 6·2, 7·4, 7·6, 5·9, 4·8	6·5	6	5·2, 6·8, 6·8, 6·3, 5·9, 6·6	6·3
Controls, no water	5	6·6, 6·2, 8·6, 5·6, 5·4	6·5			

Discussion.

In rats water given per os is absorbed as usual under the influence of pitressin, but is only excreted at a greatly diminished rate by the kidney. This water, which forms 5 p.c. of the body weight of the animal, in a period of about ½ hour is absorbed and either stored in the blood and

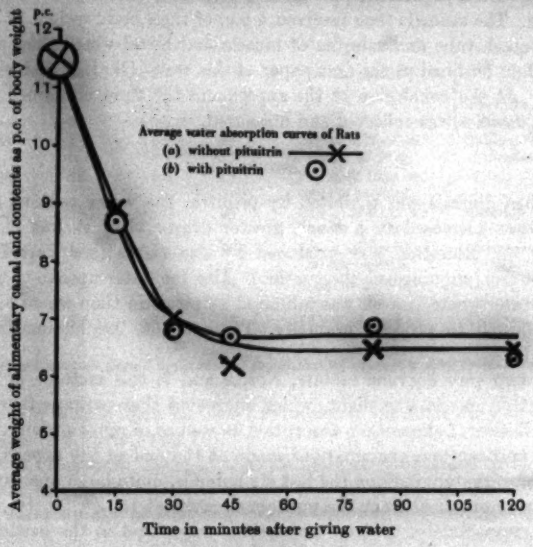

Fig. 1.

tissues of the animal or excreted by some channel other than the kidney. The only channel not under direct observation is the respiratory tract. As a precaution, therefore, a number of animals were weighed some hours after giving the water to make sure that such water as had not been excreted by the kidneys was still present in the body. There was indeed a loss of weight, but this did not account for the greater part of the water which was evidently stored in the blood and tissues of the animal.

Since it is difficult to take tissue and blood samples from a small animal such as the rat it was decided to continue our investigations on water distribution in another animal, the rabbit.

B. The storage of water in the muscle, skin, blood and serous cavities of rabbits during the inhibition of a water diuresis by pituitary hormone.

Experimental procedure.

0·2–0·4 c.c. of pitressin was given 30–45 min. before water administration. The animals then received 4 p.c. of their body weight of water by stomach tube and samples of muscle and blood were taken by the procedure outlined in the first paper of this series [Heller and Smirk, 1932]. At the conclusion of the experiment the fluid contained in the serous cavities was collected and measured.

Results.

When diuresis was inhibited by pituitrin the water content of the blood was increased to a much greater degree than it was in cases where the inhibition was produced by anæsthesia (see Part IV) or laparotomy (unpublished observation). Also the water uptake of muscle was greater where diuresis was inhibited by pituitrin than where diuresis was allowed to proceed normally. These results are summarized in Table II.

In two very nervous rabbits, Nos. 6 and 7, this increase in water content of muscle was slight, which suggested that perhaps there was an inhibition of alimentary absorption as well as of renal activity.

In post-mortem examinations made at the end of the experiments, but unfortunately only on the last six animals, quite large quantities of fluid were obtained from the peritoneal cavities: 15, 20, 20, 10, 4 and 14 c.c. respectively. Not only was there more fluid in the peritoneum but also there was more in the pleural and pericardial cavities than had been found in six of the rabbits where no pituitrin had been given and an examination of these cavities made at the conclusion of the experiment. In these latter animals there had been no inhibition of diuresis.

It is clear then when water is absorbed from the alimentary tract and fails to be excreted by the kidneys there is a greater increase in the water content of muscle and of serous cavities. Our methods, therefore, detect this water when it is absorbed, and our previous and subsequent observations concerning the absence of an appreciable increase in the water content of muscle must be regarded as strong evidence that when diuresis has been inhibited without an appreciable increase in the water

TABLE II.

Animal No.	3-hour output of urine: Normal c.c.	3-hour output of urine: Under pituitrin c.c.	Increase in p.c. water of muscle	Degree of blood dilution (as a percentage of the initial concentration)	Post-mortem examination after 3 hours	Notes
1	57	1	+3·0	—	None made	Previous diet of oats, 0·2 c.c. pitressin
2	42	11	+3·2	—		
3	48	10	+4·4	—		
4	84	18	+2·7	—		
5	76	57	+2·5	—		
6	—	10	+0·5	93·5		Previous diet of cabbage, 0·3 c.c. infundin
7	—	30	+0·4	94		
8	89	11	+1·2	91	15 c.c. of fluid in serous cavities	Cabbage diet, 0·5 c.c. infundin
9	98	22	+1·8	88	20 c.c. of fluid in serous cavities	—
10	92	10	+2·0	95	20 c.c. of fluid in serous cavities	Cabbage diet, 0·2 c.c. pitressin
11	66	9	+1·9	92	10 c.c. of fluid in serous cavities	Cabbage diet, 0·2 c.c. pitressin P.D.
12	49*	4	−0·2	94	4 c.c. of fluid in serous cavities	Cabbage diet, 0·4 c.c. pitressin P.D.
13	67*	11	+1·3	88	14 c.c. of fluid in serous cavities	Cabbage diet, 0·4 c.c. pitressin P.D.

* These two animals passed copious fluid stools shortly after receiving their doses of water.

content of muscle there has been a reduction in the alimentary absorption of water (see Parts I and IV).

It is clear from Table II that an increase is obtained in the water content of longissimus dorsi muscle, but the degree of increase varies. The water uptake of a second muscle—the vastus externus—was next studied (Table III), to obtain an idea of how far the changes in the water content of one muscle were an index of changes in the musculature as a whole.

TABLE III. The uptake of water by two different muscles under the influence of pitressin.

Exp. No.	Increase in p.c. water of two muscles 90 min. after giving water: Longissimus dorsi	Vastus externus
1	+2·9	+1·9
2	+2·0	+1·6
3	+1·4	+1·4
4	+1·3	+1·6
5	+3·1	+2·0
Average	+2·1	+1·7

As a control observation the influence of pitressin alone on the water content of muscle was investigated in five animals. Beyond the fact that no additional water was given the experimental conditions were unaltered.

The changes in the water content of muscle were + 0·6, + 1·4, + 0·3, − 0·8, − 0·2, averaging + 0·3 p.c.

The corresponding control in which water but no pituitrin was given has been described in the first paper of this series.

Because the removal of as little as 2 c.c. of blood may be associated with a blood dilution in the subsequent sample [Smirk, 1932] and also because the loss of blood from muscle sampling though slight is indefinite, it has been thought desirable to make a few separate experiments on the blood dilution in animals where no muscle samples were taken.

The results are divided into two groups (Tables IV and V) which differ in the lengths of the time periods between giving pitressin and water and between giving water and taking the second blood sample. To the first group (Table IV), in which a considerable blood dilution is

TABLE IV. Animals received 0·2 c.c. pitressin ½–¾ hour before giving water and the second blood sample was taken 1½ hours after giving 4 p.c. body weight of water.

Exp. No.	Degree of blood dilution expressed as a percentage of the initial concentration		Urinary output in 1½ hours after water
	By hæmatocrit	By hæmoglobin	c.c.
1	93	86	0·0
2	—	96·5	1·0
3	98·5	96·6	5·0
4	92·2	87·8	4·5

TABLE V. Animals received 0·2 c.c. pitressin at least 2 hours before giving water and the second blood sample was taken 2 hours after giving 4 p.c. body weight of water.

Exp. No.	Degree of blood dilution expressed as a percentage of the initial concentration		Urinary output in 2 hours after water
	By hæmatocrit	By hæmoglobin	c.c.
1	98·8	101	0·0
2	101·4	105·4	4·0
3	100	99·0	0·0
4	107·3	100·4	5·5
5	110	104·8	6·0

the rule, may be added the results of eight experiments from Table II, in which the experimental conditions were similar. In the second group, of five experiments in all, there is no regular diminution in either the hæmatocrit reading or the hæmoglobin percentage. The only essential difference appeared to be that pitressin was given a longer time before the water in the experiments where there was no regular blood dilution.

For the present we do not intend to investigate this time difference in the action of pitressin or to confirm whether it is indeed a regular phenomenon. It may indicate some mechanism by which the partition of water between blood and tissues can be altered. There did not appear to be any defect in alimentary absorption.

Magnus and his school have investigated the part played by the skin in the storage of water and salt. Since water is normally stored in skin [see also Heller and Smirk, 1932] we decided to investigate the changes in water content after giving pituitrin and water.

A small piece of skin was excised from the back with the aid of local subcutaneous anæsthesia. The five animals were given 0·2 c.c. of pituitrin and then 4 p.c. body weight of water by stomach tube. After 90 min. a second skin sample was removed in the same way and the animals were killed. This procedure was repeated in a series of another five animals in which water but no pituitrin was given. At 105 min. after giving pituitrin and 90 min. after giving water the changes in the water content of muscle were: − 2·5, − 1·0, − 0·8, − 0·3, − 1·2, average − 1·2 p.c. In the control animals which received water only the changes were: + 3·5, + 1·1, − 1·6, + 0·6, + 5·5, average + 1·8 p.c. It appears that the water content of skin is diminished in the animals which received pituitrin injections despite the administration of additional water.

The influence of pituitrin upon the extrarenal water loss. In view of the marked variations in the extrarenal loss of water which followed changes in the temperature of the surrounding air (Part II) we considered it worth while to determine whether there was any appreciable change in the extrarenal water elimination when a water diuresis is inhibited by pituitrin.

In five rabbits the non-fæcal extrarenal loss of water was determined by weighing the animals before and 4 hours after a dose of water, and then again before and 4 hours after a dose of water together with 1 unit of pituitrin subcutaneously. The room temperature was $17 \pm 1°$ C. on both occasions.

The losses of water were 13·5, 16, 10, 8 and 10·5 g. in 4 hours, average 11·6, when the animals received water only, and 27·5, 16, 15, 31·5, and 30·5 g. in 4 hours, average 24·1, when the diuresis was inhibited by pituitrin.

Presumably, since rabbits do not sweat, this increased loss of water takes place through the lungs. The increased loss may be caused either by an increase in the moistness of the respiratory passages or by an increase in the pulmonary ventilation.

It is clear, however, that although the extrarenal water elimination appears to be greater after pituitrin yet the difference in the quantities of water lost is in no case sufficient to account for the inhibition of renal activity. More detailed confirmation of this point would not, however, be relevant to our present work.

Discussion.

It will be seen from the results obtained in rabbits (see Tables II and IV) that when pituitrin is injected subcutaneously ½ hour before the administration of a large dose of water so that the diuresis which normally results is prevented, there is usually a definite hydræmia. The water content of the muscles is also increased (Tables II and III), and the degree of this is much greater than that met when diuresis is allowed to proceed normally. In addition in all those cases where an examination has been made there was an increase in the fluid content of the serous cavities, so that whereas normally it is difficult to collect more than 1 or 2 c.c. of fluid, one is now able to obtain as much as 15 c.c. or 20 c.c. As a parallel observation one of us [Heller, 1930] has previously reported the distension of the lymph spaces in frogs. It was suggested by Rees [1920] and by Koref and Mauntner [1926] that failure of alimentary absorption might account for a part of the inhibitory action of pituitrin. In the rabbit it is clear, however, that there is a definite excess of water present in both musculature and blood. We suggest that, since inhibition is present when there is an excess of absorbed water to be disposed of, it is not likely that changes in the water absorption rate play any considerable part in the pituitrin inhibition.

It has been argued that the excess of water which has been shown to be present in the blood may not be free but in some physico-chemical way "bound." This, however, is unlikely, since there is regularly an increased fluid content of the serous cavities which would appear to result from the increased transudation following a dilution of blood colloids.

An increased "avidity" of plasma for water would hardly be associated with increased transudation from the capillaries into the body cavities, and any increased "avidity" of tissues would surely need to prevent blood dilution before it could be cited as a direct cause of diminished renal activity. Heller's work on frogs [1930] yielded no evidence of a direct action of pituitrin on the water metabolism of living muscle, or upon the physico-chemical properties of tissue proteins. Heller was able to show that the increase of weight in frogs under the influence of pituitrin is not decreased by a rise in body temperature.

It appears likely therefore that the mechanism of the inhibition of water diuresis by pituitary hormone is renal and not extrarenal. There remain facts which would be difficult to explain if it were assumed that all the changes in water metabolism after pituitrin were the result of the inhibition of renal activity.

From Table III it is clear that the water distribution and storage is not uniform throughout the musculature, and similar differences in the water uptake of different muscles have been reported by Baer [1926] and Tashiro [1926]. The results obtained on skin—in which the water content was actually reduced after pituitrin and water administration—make it clear that a complete picture of the water distribution could only be obtained by the analysis of several samples of every tissue and by the elimination of errors due to changing vascularity.

The fall in the water content of skin may very well be due to its lessened blood content, the percentage water content of blood being greater than that of muscle. If corrections could be introduced which eliminated this factor, it is possible that the water content of the actual skin substance would be increased. The actual amounts of water involved are in any case small since the skin forms only 12 p.c. of the body weight.

Poulsson [1930] concludes that the reduced output of water after pituitrin is due to an increased reabsorption of water in the tubules, since the action of pituitrin in inhibiting diuresis is not accompanied by any appreciable change in the calculated amount of glomerular filtrate.

His calculations of the amounts of glomerular filtrate require, however, the assumption that all the creatinine and sulphate excreted in the urine is filtered by the glomeruli and reaches its urinary concentration by water absorption in the tubules.

The internal localization of function in the kidney is a subject of great difficulty, and the work of Starling and Verney [1925] is not in agreement with the assumption which Poulsson and others have used to calculate the amount of glomerular filtrate.

Summary.

1. In rats the alimentary absorption rate for a 5 p.c. body weight dose of water is uninfluenced by giving 1 unit of pitressin per 100 g. rat subcutaneously. This dose of pitressin considerably reduces the formation of urine.

2. In rabbits receiving 0·2–0·4 c.c. of pitressin and 4 p.c. body weight of water, there is reduced urine formation and increased water storage in the blood, muscle and serous cavities.

3. The nature of the anti-diuretic action of pituitary hormone is discussed in the light of these observations.

We wish to thank Prof. T. R. Elliott for his helpful criticism and advice.

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Part IV. The influence of anæsthetics and hypnotics on the absorption and excretion of water.

Introduction.

It has been demonstrated by many workers, Schroeder [1888], Frey [1907], Molitor and Pick [1925, 1926, 1927], Smith and McClosky [1925], Stehle and Bourne [1928], Buschke [1928], Fee [1928], Kugel [1929], Bonsmann [1930], that the excretion by the kidneys of a large dose of water administered by stomach tube is rarely normal in an anæsthetized animal. The effect of anæsthesia appears to depend upon the nature of the anæsthetic, upon the depth of anæsthesia and also upon

the animal used. With most anæsthetics the urinary output is diminished, with a few it is increased. The school of Pick conclude that in general the influence of anæsthetics results from their action upon the central nervous system. Narcotics which depress first the higher centres such as paraldehyde, chloralose, avertin, and alcohol tend to increase the outflow of urine; those which first depress the mid-brain areas tend to diminish the activity of the kidney (luminal, chloretone). It is supposed by them that the normal control of diuresis is through a diuretic centre in the mid-brain: the depression of its activity diminishes renal activity; the depression of higher control centres with release of its activity is said to increase urinary outflow.

Though opinions may differ as to the precise way in which anæsthesia influences the output of urine there is little doubt that a number of substances of differing chemical structures but having in common the property of producing narcosis have also in common the property of reducing the urinary outflow. The object of this paper is to determine whether some alteration in the kidney or tissues is responsible for this anti-diuretic action or if alterations in alimentary absorption play a part.

In a few experiments performed by us on rabbits anæsthetized with ether it was observed that a dose of about 4 p.c. of the animal's body weight of water produced little or no increase in the output of urine. But samples of muscle in these animals showed only slight increases in their water content. Now in rabbits where renal activity was prevented by pituitrin but where there was every evidence of water absorption the extra water given was readily detected in muscle samples (see Part III). Therefore, the absence of any considerable increase in the water content of muscle in these anæsthetized rabbits suggested to us that a part at least of this apparent inhibition of renal activity might be of alimentary origin and due to the non-absorption of the water given.

For this reason we decided to investigate the matter more accurately, using the statistical method of studying alimentary absorption in rats already described in Part I of this series [Heller and Smirk, 1932]. The rat is particularly suitable for this investigation since we have shown that water absorption is practically complete before the onset of diuresis. It is, therefore, possible to investigate also the effect of anæsthesia on diuresis as distinct from absorption by administering the anæsthetic at a time when water absorption is almost complete and diuresis is only just beginning.

Experimental procedure.

Rabbits weighing about 2 kg. were used and a uniform dose of 75 c.c. of water was given. The methods employed were light ether anæsthesia, deep chloretone anæsthesia and light chloretone anæsthesia. The water and chloretone were given by stomach tube, the ether by inhalation.

The procedure for rats has been exactly as described in Part I, chloroform and ether were given by inhalation; paraldehyde, luminal, urethane and chloralose were given subcutaneously. The doses and previous diet are stated with the protocols. Body temperatures were measured per rectum.

Results.

Preliminary experiments on rabbits. Four animals received ether. Under ether their urinary outputs for periods of 4, 4, 3 and 4 hours were 2, 19, 5 and 0·5 c.c. as compared with their normal outputs of 29, 35, 36 and 52 c.c. for corresponding time periods. The increases observed in the percentage water content of muscle 1½ hours after giving water were only + 0·8, + 0·9, 0·0, + 0·8, averaging + 0·6 as against an average increase of + 2·0 p.c. where diuresis is inhibited to a corresponding degree by pituitrin (see Part III).

Four experiments, two with light chloretone anæsthesia and two with deep chloretone anæsthesia, gave a similar absence of appreciable change in the water content of muscle and no increase in the water content of liver (determinations made on 16 samples of muscle and 18 samples of liver). The technique of removing liver samples and the influence of the procedure of laparotomy upon absorption and diuresis will be described in a subsequent paper. The urinary output was also inappreciable.

Although the chloretone experiments are complicated by the intestinal method of administration as well as by laparotomy, the absence of expected increases in the water content of the tissues examined appears to be a justification for further work.

I. *The influence of anæsthetics on water absorption in rats.* It will be clear from the following results that the rate of absorption of water from the alimentary canal of rats is greatly diminished by ether (Table I)

TABLE I. Ether. Light ether anæsthesia with usually a positive corneal reflex. Deprived of water overnight.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
60 min.	5	9·0, 10·2, 9·5, 7·3, 8·7	9·0
Controls, no extra water	5	5·3, 5·8, 6·7, 6·8, 7·0	6·3

and chloroform (Table II); slightly diminished by luminal (Table III), paraldehyde (Table IV) and urethane (Table V), and hardly diminished by chloralose (Table VI). The inhibition of absorption produced by ether is unaltered by physostigmine (Table VII).

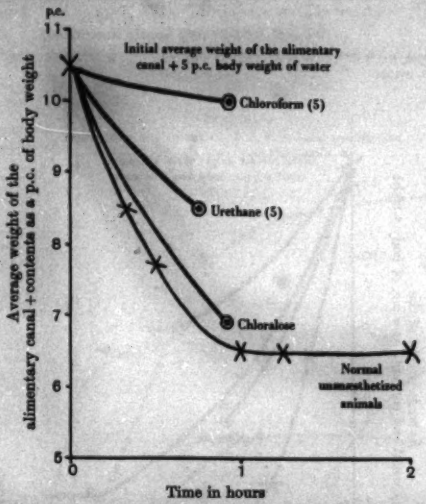

Fig. 2. The influence of chloroform, urethane and chloralose upon the alimentary absorption of water. (The animals were allowed access to water overnight.)

Table II. Chloroform. Light chloroform anæsthesia with usually a positive corneal reflex. Allowed water up to the time of the experiment.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
55 min.	5	10·2, 8·3, 10·7, 10·4, 10·4	10·0

Table III. Luminal. (0·015 g. of sodium luminal per 100 g. rat deprived of water overnight.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
30 min.	5	8·5, 8·8, 8·3, 11·5, 9·9	9·4
45 ,,	5	7·2, 7·7, 5·2, 9·5, 9·6	7·8
90 ,,	6	5·0, 5·8, 5·9, 7·6, 8·4, 6·8	6·6
Controls, no extra water	5	5·3, 5·8, 6·7, 6·8, 7·0	6·3

TABLE IV. Paraldehyde. (0·2 g. of paraldehyde subcutaneously per 100 g. rat. Deprived of water overnight.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
30 min.	4	8·4, 6·4, 7·2, 7·5	7·4
45 „	5	8·9, 7·5, 7·3, 7·4, 7·0	—
		5·2 (very lightly narcotized)	7·6
Controls, no extra water	3	6·3, 5·0, 5·1	—

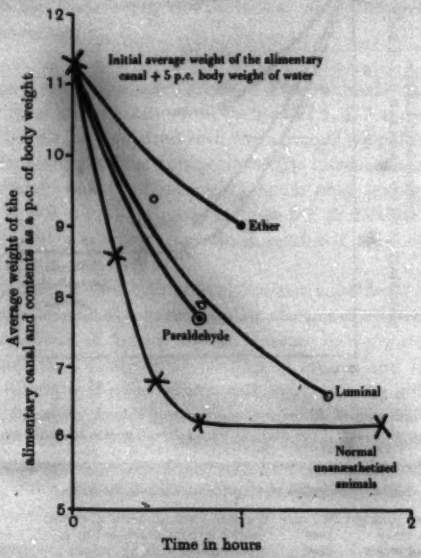

Fig. 3. The influence of ether, paraldehyde and luminal upon the alimentary absorption of water. (The animals were previously deprived of water overnight.)

TABLE V. Urethane. 0·12 g. of urethane per 100 g. rat. Water allowed up to the time of the experiment.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
45 min.	5	8·2, 7·4, 7·1, 8·9, 10·8	8·5
Controls, no extra water	7	6·3, 5·0, 5·1, 5·5, 6·8, 6·6, 5·5	5·7

TABLE VI. Chloralose. Two animals had 0·01 and one animal 0·008 g. chloralose per 100 g. rat. Water allowed up to the time of the experiment. Because of the relative insolubility of chloralose larger quantities of fluid had to be injected. The controls were given the same amount of 0·2 p.c. NaCl (2 c.c.) subcutaneously.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
55 min.	5	6·0, 6·8, 8·9, 6·5, 7·0	6·9
Controls, no extra water	7	6·3, 5·0, 5·1, 5·5, 6·8, 6·0, 5·5	5·7

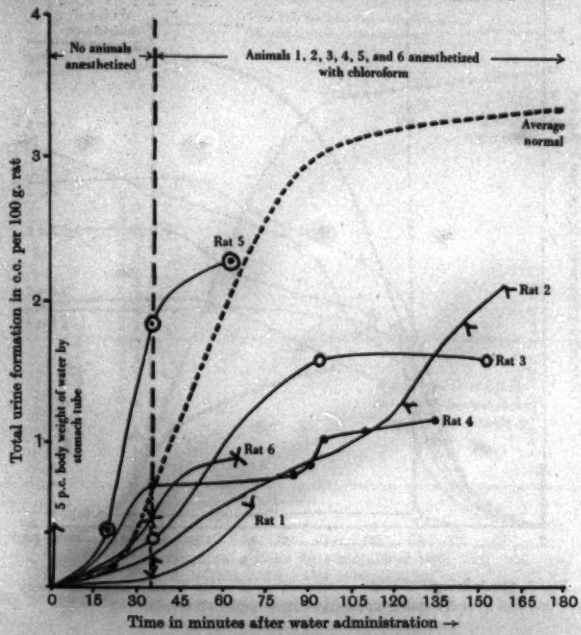
No animals anæsthetized
Animals 1, 2, 3, 4, 5, and 6 anæsthetized with chloroform
Average normal
Rat 5
Rat 2
Rat 3
Rat 4
Rat 6
Rat 1
5 p.c. body weight of water by stomach tube
Total urine formation in c.c. per 100 g. rat
Time in minutes after water administration →
0 15 30 45 60 75 90 105 120 135 150 165 180
1 2 3 4

Fig. 4. The influence of chloroform anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

TABLE VII. Ether and physostigmine. Light ether anæsthesia with usually a positive corneal reflex. Deprived of water overnight. (Record of the dose of physostigmine lost.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
60 min.	5	9·6, 9·1, 9·3, 8·0, 8·0	8·8

The results are summarized in graphical form on Figs. 2 and 3, on which the normal absorption curves described in Part I are reproduced. Since it is not possible with injected drugs to obtain much uniformity in the degree of narcosis except by repeated trials on the same animal an increase in the variability of results is to be expected.

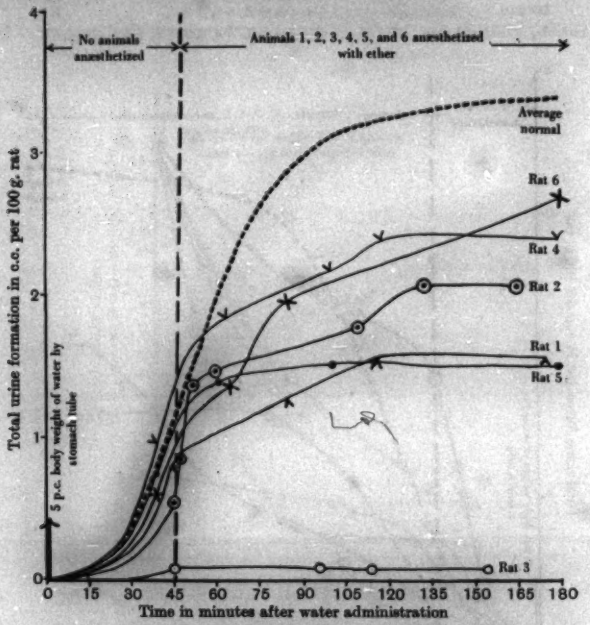

Fig. 5. The influence of ether anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

All rats used in these experiments were fed previously on bread and milk for a period of 3 days.

II. *The influences of chloroform and ether upon water diuresis when absorption is complete.* If water is given to an animal and a sufficient time for water absorption is allowed before subsequently administering the anæsthetic, it has been found that a marked inhibition of diuresis is still obtained. This is clearly seen on Figs. 4 and 5, where the diuresis in

each rat which was anæsthetized falls rapidly below the average normal output of the controls. With the exception that no anæsthetic was given the control rats were similarly treated. Figs. 6 and 7 give the average diuresis curves for the anæsthetized and normal rats and indicate the times at which the water absorption may be assumed to have taken place.

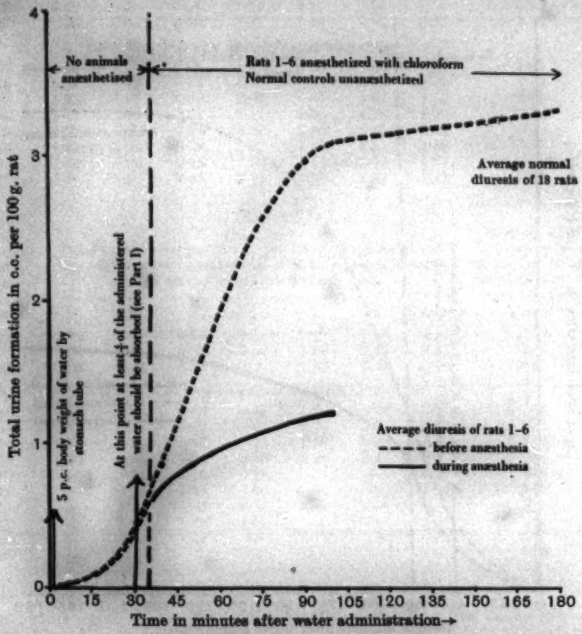
No animals anæsthetized
Rats 1–6 anæsthetized with chloroform
Normal controls unanæsthetized
Average normal diuresis of 18 rats
Total urine formation in c.c. per 100 g. rat
5 p.c. body weight of water by stomach tube
At this point at least ½ of the administered water should be absorbed (see Part I)
Average diuresis of rats 1–6
before anæsthesia
during anæsthesia
0
1
2
3
4
0 15 30 45 60 75 90 105 120 135 150 165 180
Time in minutes after water administration

Fig. 6. The influence of chloroform anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

From Tables VIII and IX it will be seen that before an anæsthetic was given the average urinary outputs of the two sets of six animals is approximately equal to that of the eighteen normal controls. But during the period when the rats were anæsthetized with ether and chloroform respectively their urinary output fell markedly below that attained by the controls.

Thus at the end of 35 min. the average total urinary output was 0·62 c.c. per 100 g. rat for the normal controls and 0·60 c.c. for the animals which were to be anæsthetized subsequently.

In the next 65 min. the average total output of the normal controls was 2·48, but of the rats anæsthetized with chloroform only 0·61 c.c. per 100 g. rat.

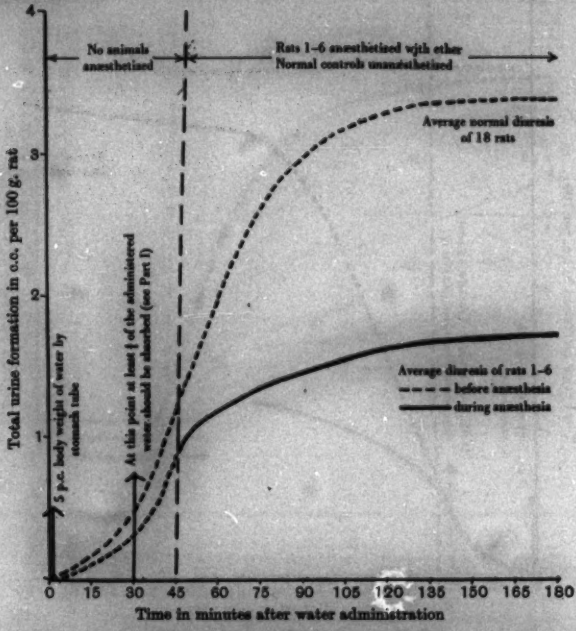

Fig. 7. The influence of ether anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

Similar results were obtained using ether anæsthesia.

The water absorption curves for rats in which the previous treatment was similar and which were of the identical breed (Part I) show that water absorption is practically complete within 30 min. after the administration of a 5 p.c. of body weight dose of water by stomach tube.

TABLE VIII.

Time after water administration (min.)	Total urinary output in c.c. per 100 g. body weight of rats which were anæsthetized with ether 45 min. after giving water by stomach tube							Average total urinary output in c.c. per 100 g. rat of 18 normal controls (no anæsthetic given)
	1	2	3	4	5	6	Average	
20	0·13	0·09	0·00	0·26	0·22	0·17	0·15	0·23
40	0·45	0·30	0·04	1·00	0·80	0·66	0·54	0·93
45	0·78	0·54	0·08	1·37	1·10	0·93	0·80	1·17
	Rats Nos. 1–6 now anæsthetized with ether							
50	0·92	1·20	0·08	1·57	1·22	1·08	1·01	1·43
60	1·04	1·46	0·08	1·79	1·37	1·26	1·17	1·95
80	1·23	1·58	0·08	2·00	1·47	1·81	1·36	2·75
100	1·42	1·70	0·08	2·17	1·50	2·10	1·49	3·10
120	1·53	1·88	0·08	2·38	1·50	2·26	1·61	—
140	1·53	2·06	0·08	2·38	1·50	2·41	1·66	—
160	1·54	2·06	0·08	2·38	1·50	2·54	1·68	—
180	1·54	2·06	0·08	2·38	1·50	2·67	1·71	3·35

TABLE IX.

Time after water administration (min.)	Total urinary output in c.c. per 100 g. body weight of rats which were anæsthetized with chloroform 35 min. after giving water by stomach tube							Average total urinary output in c.c. per 100 g. rat of 18 controls (no anæsthetic given)
	1	2	3	4	5	6	Average	
20	0·00	0·04	0·08	0·19	0·40	0·12	0·14	0·23
30	0·02	0·11	0·20	0·50	1·28	0·33	0·41	0·40
35	0·06	0·20	0·32	0·70	1·83	0·47	0·60	0·62
	Rats Nos. 1–6 now anæsthetized with chloroform							
40	0·12	0·30	0·46	0·75	2·00	0·60	0·71	0·93
45	0·17	0·37	0·60	0·77	2·10	0·71	0·79	1·17
50	0·29	0·43	0·72	0·78	2·16	0·78	0·86	1·43
60	0·34	0·54	1·01	0·79	2·26	0·87	0·97	1·95
80	—	0·74	1·40	0·79	—	—	0·98	2·75
100	—	0·98	1·58	1·06	—	—	1·21	3·10
120	—	1·22	1·58	1·12	—	—	—	—
140	—	1·74	1·58	1·17	—	—	—	—
160	—	2·10	1·58	—	—	—	—	—
180	—	—	—	—	—	—	—	3·35

It is clear, therefore, that the results represent the influence of these anæsthetics upon the urinary output after absorption has taken place.

The extrarenal loss of water determined in rats anæsthetized with ether amounted to 3·55, 0·99, 2·97, 3·4, 1·16 and 3·38 per 100 g. rat in 3 hours. In two animals anæsthetized with chloroform it was 2·75 and 3·90 g. per 100 g. rat in 159 and 152 min. respectively. This is not in excess of normal.

The body temperature of the rats fell during anæsthesia, but for reasons described in a previous paper (Part II) it was thought inadvisable to apply heat externally. In any case the inhibition of diuresis takes place before there has been time for any appreciable loss of water or fall in temperature.

Discussion.

In rats the delay in intestinal absorption of water produced by ether and chloroform is so great that this alone would explain the absence of diuresis. But it has also been shown, that even when the induction of anæsthesia is postponed and time is allowed for water absorption there is still a marked inhibition of diuresis. The inhibitory actions of ether and chloroform are therefore twofold: on the alimentary canal delaying absorption and after absorption preventing excretion.

The experiments on rabbits suggest that anæsthetics may also tend to diminish the alimentary absorption in this animal.

In studying the inter-relationship of the nervous system and kidneys and in all diuresis experiments involving the administration of water by the alimentary canal it is clearly necessary to distinguish between enteral and renal phenomena. The parts played by these factors vary and must be separately assessed for each animal and anæsthetic used or a final interpretation of the results may be obscured.

Summary.

In rats: (1) The alimentary absorption of water is delayed to a marked degree by ether and chloroform, to a slighter degree by luminal, paraldehyde and urethane and is not appreciably delayed by chloralose.

(2) Diuresis is inhibited by ether and chloroform even when adequate time for water absorption is allowed prior to the induction of anæsthesia.

(3) The inhibition of diuresis by ether and chloroform is not caused by any increased extrarenal water elimination.

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AN ANTI-GROWTH PRINCIPLE DERIVED FROM THE PARATHYROID GLAND.

By M. H. B. ROBINSON and J. H. THOMPSON.

(From the Physiological Laboratories of the University of London, King's College, and the Royal College of Surgeons.)

During the course of another investigation the existence in the parathyroid glands of a principle other than that controlling calcium metabolism was suggested. Moreover, preliminary experiments involving the injection of "parathormone" in rats over long periods demonstrated, among other things, some retardation of growth.

That the parathyroid glands play some part in body growth was further suggested by the somewhat sparse literature of this subject. Hansman [1930] has recently performed thyro-parathyroidectomy operations resulting in a marked gain in weight which, he maintains, is to be regarded as a manifestation of parathyroid imbalance, since the control experiments definitely rule out the possibility of these symptoms being due to hypothyroidism.

Hyperparathyroidism in children is reported as being associated with emaciation and decrease in body weight and failure to grow [Hurst and Cosin, 1931; Pemberton and Geddie, 1930].

It may also be of significance that the oxyphil cells of the parathyroid gland do not develop until the rate of growth begins to slow. In man they do not appear until the age of about adolescence, while hyperplasia of the parathyroid glands in aged individuals has been reported [Kurikawa, 1925].

Another fact possibly having some bearing on the subject is that parathyroid glands have not been found in fish and, as is well known, fish never cease to grow.

Therefore, the problem whether the parathyroid glands have some function relating to growth seemed worthy of investigation, and extracts of parathyroid glands free from the blood calcium raising factor (Collip) were prepared and the effects of their administration in animals observed.

The present paper presents the results thus obtained upon the growth rate of the animals. A preliminary paper has already been made by one of us (J. H. T.) [1931].

Methods of preparation.

A large number of preparations from ox parathyroid glands have been made. Many of these have been inactive in relation to animal growth, and most of them are not mentioned in this paper. The majority of the active extracts are modifications of the original method of preparation, details of which are given below. A fuller description of the methods of preparation and of the chemical properties of the extracts will be given elsewhere.

We are much indebted to Messrs Allen and Hanbury's, Ltd., for preparing many of these extracts, and for most generously supplying a large quantity of the original extract for the routine work.

Mr N. Evers has kindly supplied us with the following details of the methods of preparation of the extracts used for routine work.

Extract 1. 400 g. of parathyroid glands were freed from adhering fat, thoroughly minced and then mixed with a cooled mixture of 300 c.c. of concentrated sulphuric acid, 1800 c.c. of water and 900 c.c. of 95 p.c. alcohol. 700 c.c. of benzene were then added and the whole shaken for 48 hours. The gland residue was then strained off and the benzene separated from the liquor after the latter had been standing for some hours. The acid-alcoholic liquid was then neutralized by the gradual addition of strong sodium hydroxide solution until the *p*H value was about 4·8. Alcohol, 95 p.c., was then added to a concentration of 75 p.c. by volume and the mixture kept in a cool place for 16 hours to allow the sodium sulphate to crystallize out. The insoluble matters were filtered off and the filtrate was evaporated under reduced pressure to a volume such that 1 c.c. was equivalent to 2 g. of original gland tissue. The *p*H value of the liquid was adjusted to 4·8, and after allowing the product to stand until it no longer deposited it was filtered through a sterilizing Seitz filter.

Extract 2. Sodium sulphate was added to an extract made by the above process until the latter was saturated. The mixture was allowed to stand overnight and the salted out material was filtered off. It was redissolved in a smaller volume of water at *p*H 4·8 and again salted out. The combined filtrates were freed from sodium sulphate by the addition of alcohol to a final concentration of 75 p.c. by volume, cooled to about 5° C. and filtered at this temperature.

The alcohol was removed by distillation under reduced pressure and the volume of aqueous residue adjusted to its original value.

Extracts treated with hydrogen peroxide. 1 c.c of "100 volume" hydrogen peroxide solution was added to 300 c.c. of an active extract, the *p*H of which was about 4·8, and the mixture kept in an incubator at 22° C. overnight. The excess of hydrogen peroxide was removed by the addition of manganese dioxide which was filtered off when the required action had taken place.

Various fractions have been prepared of which a few are detailed below.

Extract No.	Method of preparation	Total solids p.c.	Ash p.c.
3	As extract 1 but heated with acid alcohol	7·4	1·3
4	Extract 3 treated with charcoal	—	—
5	Filtrate from treatment of extract 3 with 80 p.c. alcohol	—	—
6	Extract 5 treated with charcoal	—	—
7	Extract 3 treated with hydrogen peroxide	—	—
8	Precipitate obtained during evaporation of extract 5	2·50	0·65
9	Extract 3 autoclaved	8·0	1·70
10	Residue, from extraction with acid 80 p.c. alcohol, extracted with acid 30 p.c. alcohol	0·7	0·3
11	Extraction of acetone soluble matter from glands with acid alcohol	—	—
12	Alkaline extract of glands	6·0	0·55
13	Proteolytic digestion of glands	—	—
14	Proteolytic digestion of lean veal	—	—

All extracts used in this series of experiments have been free from all antiseptics, adjusted to *p*H 7, and are protein free.

Methods of administration. In all cases, unless otherwise stated, the extracts have been given by subcutaneous injection once daily.

An ordinary diet has been given to all animals, and in most instances the experimental animals and their controls have lived together in the same cages.

No local reaction has ever resulted from injecting the extracts detailed in this paper, although considerable reaction occurred with earlier extracts due to failure of adjusting the solution to *p*H 7.

Results.

Retardation of growth has been successfully obtained in all the different kinds of animals used, viz. rabbits, rats, guinea-pigs, tadpoles and axolotls. The present paper deals with rabbits and rats. In no instance have any harmful effects been observed with the dosages used. No sickness, vomiting, diarrhœa, convulsions, nor any signs of hypercalcæmia have been observed. The animals have remained apparently healthy, feeding and exercising in a perfectly normal manner. Indeed, some slight beneficial effects have been noticed, the experimental animals being less subject to intercurrent disease.

Rabbits. Fig. 1 shows the average growth curves for the first 12 months of all the rabbits having one 5 c.c. dose daily, the dose used in this investigation thus far. As will be seen the growth rate of the experimental animals begins to be decreased about 4 weeks after the beginning of the injection of extract 1. Extract 2, however, induces an earlier retardation. With a 5 c.c. dosage growth is markedly retarded, and in some instances completely inhibited for a few months.

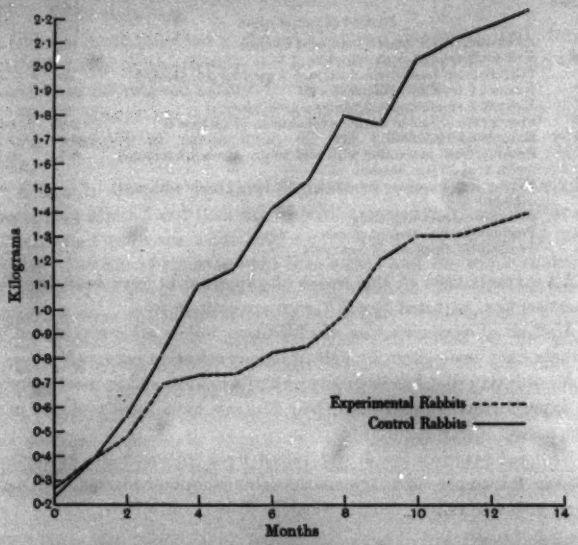

Fig. 1. Average growth curves of 26 rabbits injected with 5 c.c. of parathyroid extract daily, and of 10 control rabbits. It will be seen that at the end of 12 months there is a difference in weight of nearly 1 kg.

With smaller doses the degree of retardation varies more or less proportionately with the dosage as will be seen from Fig. 2.

Rabbits, inhibited in their growth rate by extract 1 (two of which are shown in Fig. 3), appear to develop after a few months a kind of tolerance to the extract. This is also obvious when using extract 2 over long periods of time, but the effect is a much smaller one. A few preliminary investigations show that when injections of extract 2 are

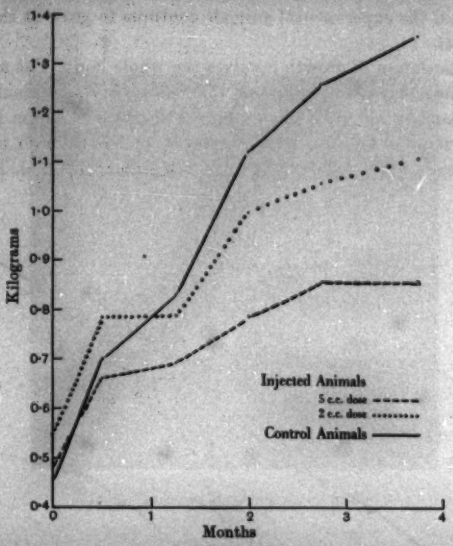

Fig. 2. Growth curves of rabbits of the same litter injected with 5 c.c. and 2 c.c. doses of parathyroid extract daily. The degree of growth retardation is approximately proportional to the dosage.

Fig. 3. Two rabbits of the same litter aged 9 months, the small one injected for 6 months with 5 c.c. parathyroid extract daily. Respective weights 1·29 kg. and 1·95 kg.

discontinued the experimental animals continue to grow at the retarded rate (Fig. 4).

The retardation of growth involves the whole body. The animals are well developed and normal except that they are much smaller in size than the controls (Fig. 3). That the growth of the skeleton is included can be seen from Fig. 5. No abnormality of the calcium metabolism has been found in the bone structure of any animals (Fig. 6). The control

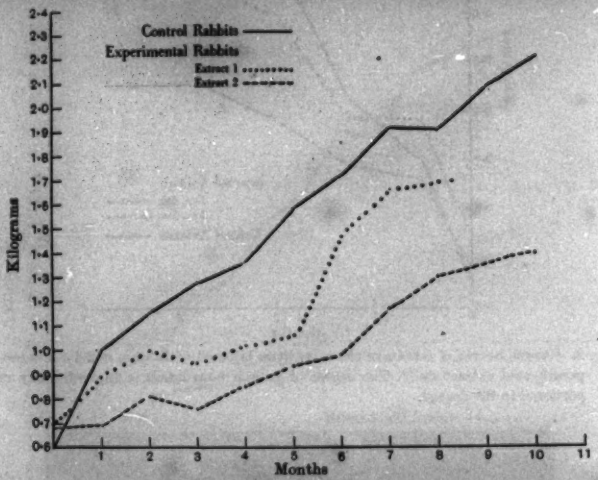

Fig. 4. Showing (a) the greater potency of extract 2 in retarding growth; (b) the tolerance developed to extract 1; (c) the continuation of the retarded growth rate after cessation of extract 2 at the seventh month.

rabbits have had equivalent doses of saline during the first 3 months, and equivalent doses of meat extract prepared in the same way as the parathyroid gland extract for the ensuing 2 months. As no effects upon the growth rate were obtained as a result of injecting it, its administration was discontinued.

More elaborate control experiments have been performed in the experiments on rats.

Rats. Extract 1 was found to be without much effect in rats. However, when more potent extracts were prepared results were readily obtained in them.

Fig. 5. Skeletons of rabbits shown in Fig. 3.

Fig. 6. Microphotograph of ground section of femur of rat injected with 6 c.c. of extract daily for 3 months to show that no decalcification of the bone has occurred.

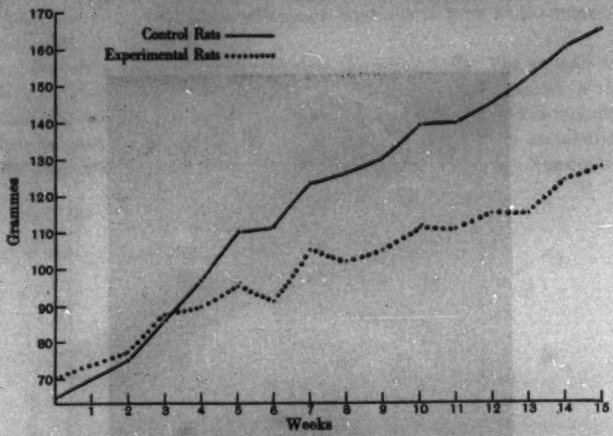

Fig. 7. Retardation of growth in rats by injection of 2 c.c. extract 2 daily. The curves represent the average weights of 24 rats.

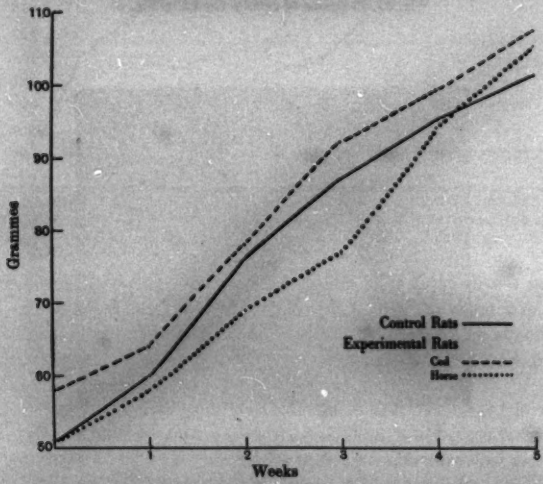

Fig. 8. The effects on growth of rats of extracts of horse muscle and of fish muscle made in a similar manner to the parathyroid extracts. 2 c.c. doses were injected. No variation from the normal growth was observed.

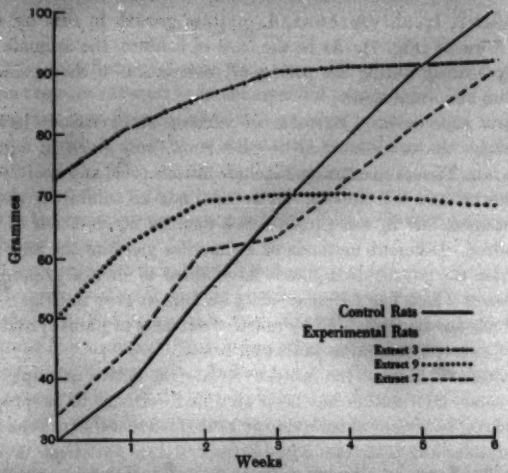

Fig. 9. Showing that autoclaving the extract does not affect its potency. The same extract, however, by washing with hydrogen peroxide has its activity destroyed.

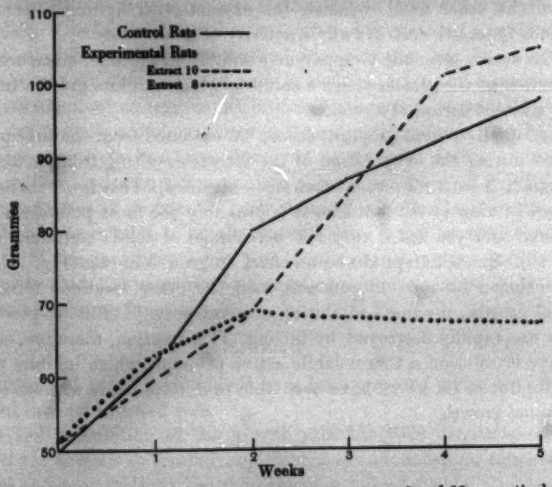

Fig. 10. The effects of injecting 2 c.c. doses daily of extracts 8 and 10 respectively upon rat growth.

Extract 2, freshly prepared, inhibits growth in rats by the end of 2 or 3 weeks (Fig. 7). As in the case of rabbits, the animals remain perfectly healthy during the period of retardation of their growth and exhibit no abnormal signs.

Control experiments. Extracts of various other tissues have been prepared by the same method of extraction, and given in equivalent doses to rats. Tissues so extracted include muscle (veal and beef), prostate gland, fœtus, and fish muscle. In no case has an inhibition of growth been obtained, but in some instances a definite acceleration of growth has resulted. Different methods of extraction yielding the anti-growth factor from the parathyroid glands have failed to elicit a principle from other tissues which has a similar effect on animal growth (Fig. 8).

Controls are also provided by inactive extracts of parathyroid glands or active extracts treated as indicated below.

Although we have so far failed to isolate the active principle, nevertheless some information has been obtained relating to its properties. For instance, boiling or autoclaving at 15 lb. for several hours the extract prepared according to extract 3 (see extract 9) does not affect its potency (Fig. 9). The extract, however, slowly loses its potency when kept at room temperature or even in a refrigerator.

On the other hand, washing the extract with hydrogen peroxide (extract 7) totally destroys all its activity (Fig. 9).

The active principle is apparently soluble in acetone, since acetone extraction of the glands yields a solution which inhibits growth in rats after a latent period of 2 weeks.

An equally potent preparation can be obtained from the precipitate formed during the evaporation of the filtrate resulting from treatment of extract 3 with 80 p.c. alcohol (see extract 8). This is of particular interest in view of the fact that it is probably the most potent fraction prepared and yet has a very low percentage of solids contained in it (Fig. 10). Extract 10 produced no effect on growth in rats (Fig. 10), but is mentioned because, in common with the other fractions retarding animal growth, it caused inhibition of plant growth. But its power to do so was rapidly destroyed by boiling. This fraction, therefore, seems to have in solution a thermolabile active principle which inhibits plant growth, but so far as we have been able to determine, is without effect on animal growth.

Discussion.

That the parathyroid glands play some rôle in growth is suggested by the observations referred to in the opening paragraphs. The difficulty in the past has been to dissociate this possible function from the well-known part which they take in regulating the calcium metabolism of the body.

No indication of any disturbance of the calcium metabolism has been observed in the experiments here recorded. Further investigation of this point is being pursued.

There is no evidence to indicate any toxicity of the preparations used. The total amount of solid matter present in the extracts is small, and it is significant in this connection that extract 8 with an extremely low solid content has a powerful inhibiting effect. None of the animals has shown any signs of toxæmia. Indeed, as already stated, many of the animals have been in slightly better physical condition than the controls. Injections daily extending over several months have failed to create any disturbance of normal health other than failure to grow. Indeed, it has been possible in several instances to keep animals constant in size and in weight for periods of 4 and 5 months.

The fact that some animals are more sensitive than others is difficult to explain. One of the difficulties encountered in reaching a conclusion is that no fully satisfactory method of extraction has been found. Extracts made in the same way vary in their potency, and occasionally are quite impotent as regards inhibition of the growth of rats. Whether this is due to some unknown factor in the extraction process, or whether the active principle has been destroyed or partially destroyed in the gland material prior to extraction we do not yet know. Nevertheless, since retardation of growth has been obtained in such a large number of animals, and no relation to any known substance in the extract or known function of the parathyroid has been demonstrable, we feel justified in concluding that the parathyroid glands contain an autacoid body with a growth-inhibiting function.

Summary.

Extracts of parathyroid glands have been prepared which have been injected into rabbits and rats.

Marked retardation of the growth rate has been demonstrated in most of the animals so treated, and reasons are advanced for concluding that an anti-growth factor exists in the parathyroid glands.

We desire to express our gratitude to Sir Arthur Keith and Prof. R. J. S. McDowall for much valuable advice and assistance, and to Mr W. MacCallum, without whose aid the earlier part of this work would have been impossible. We wish to thank too Dr W. Robson and Mr W. T. Boyd for kindly preparing some of the extracts.

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THE EFFECT OF PARATHYROID HORMONE AND OF IRRADIATED ERGOSTEROL ON CALCIUM AND PHOSPHOROUS METABOLISM IN THE RAT.

By L. I. PUGSLEY.

(From the Department of Biochemistry, McGill University, Montreal, Canada.)

I. Introduction.

The rat is commonly regarded as a species relatively refractory to the parathyroid hormone, although Tweedy and Chandler [1929] were able to show that continued large doses were lethal, and Rose and Stucky [1930] found that the serum calcium could be raised by two or three days' treatment. An increased calcium excretion has been observed by Waltner [1928], and very recently by Bülbring [1931], the calcium being presumably derived from the bones, which according to Waltner [1928] and to Lambie, Kermack and Harvey [1929] soon display osteoporosis. The object of the present research was to reinvestigate the action of parathyroid hormone in the rat, and to compare it with the action of large doses of irradiated ergosterol[1]. The latter is known to decrease calcium excretion in the fæces and increase it in the urine [Brown and Shohl, 1930; Watchorn, 1930] and the interpretation usually offered is that the absorption of calcium from the intestine is greatly increased, although according to Harris and Innes [1931] and others mobilization of calcium from the bones must also take place. Taylor, Weld, Branion and Kay [1931] affirm that in the dog the effects of overdosage with irradiated ergosterol are identical with those of parathyroid overdosage, and consider that in large doses vitamin D stimulates secretion from the parathyroid glands. It will be shown, however, that in the rat the metabolic effects of the two substances are entirely dissimilar, and recent studies in this laboratory by Selye [1932] prove that different histological changes are produced in the skeleton by these two agents.

The problem first investigated in this research was the effect of parathyroid hormone on the excretion of calcium and phosphorus in the urine and fæces. Greenwald and Gross [1926] found that the administration of parathyroid hormone to dogs led to an increased excretion of

[1] The writer is indebted to Eli Lilly and Co. for the parathormone and to Mead, Johnson and Co. for the ergosterol used in these experiments.

calcium in the urine, which was not maintained throughout the period of treatment and declined to subnormal levels in the recovery period; the phosphorus excretion in the urine followed a similar course, while the excretion of these elements in the fæces was little affected. These results have been confirmed by many workers, especially in studies of human subjects, in whom, according to Albright, Bauer, Ropes and Aub [1929] the rise in urinary calcium appears later than, and is probably secondary to, the rise in urinary phosphorus. Other references may be found in a recent review of the literature [Thomson and Collip, 1932].

II. General methods.

Albino rats of Wistar stock were used. They were kept in Hopkins' metabolism cages, and the urine and fæces were collected separately. In order to obtain sufficient urine for calcium analyses it was necessary to keep two rats (litter-mates) in a cage, and allow the urine to collect for 48 hours; the results, however, are expressed in mg. per rat per day throughout this paper. The diet used was McCollum's stock diet, except that no powdered bone was included, as it was felt undesirable to run the risk of contamination of the excreta with this ingredient; as compounded, the diet contained 0·266 p.c. phosphorus and 0·025 p.c. calcium. Male and female rats were used indifferently, and the animals weighed from 180 to 205 g. at the beginning of the experiments.

Analytical methods. The urine was ashed by a modification of the technique described by Kutz [1931] from this laboratory for dried tissue. It was evaporated to dryness in the 125 c.c. Pyrex Erlenmeyer flasks in which it was collected, and boiled for 3 or 4 hours with 3 to 5 c.c. of concentrated nitric acid (redistilled in glass to get rid of the traces of calcium usually present) until the digestion mixture was a clear straw-yellow; the nitric acid was then allowed to evaporate, and successive small amounts of nitric acid were added and evaporated until a white ash was obtained; the heating was carried out on a sand bath at about 200° C., and the whole process took about 24 hours. The ash was dissolved in HCl, calcium was determined on an aliquot portion by the method of Clark and Collip [1925], phosphorus on another portion (after heating with 10*N* H_2SO_4 to convert metaphosphate into orthophosphate, and to drive off excess HCl) by the method of Fiske and Subbarow [1925]. It was shown that known amounts of phosphate added to the urine could be quantitatively recovered by this method. The fæces were collected and dried in a silica dish and ashed in an electric muffle furnace at 500° C. for 3 hours; the ash was analysed by the method described above.

III. Experimental results.

The effect of parathyroid hormone upon the excretion of calcium and phosphorus by adult rats. The first problem studied was the effect of

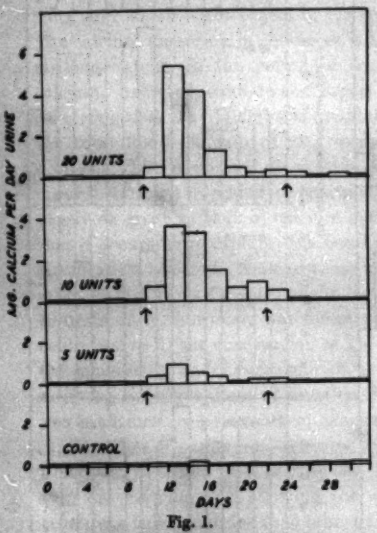

Fig. 1.

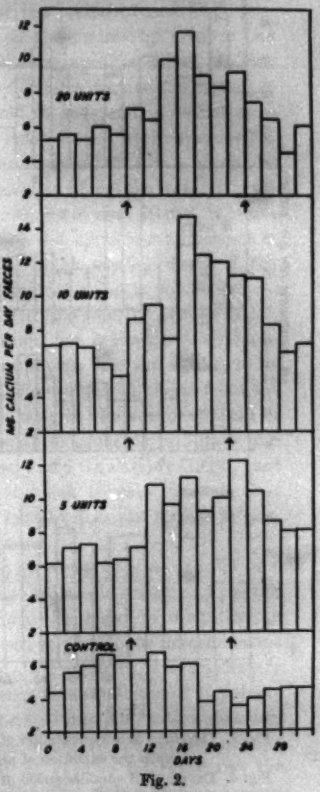

Fig. 2.

Fig. 1. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of calcium in the urine of adult rats.

Fig. 2. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of calcium in the fæces of adult rats.

varying amounts of parathyroid hormone ("Parathormone" Lilly) on the excretion of calcium and phosphorus. The experiments were divided into three periods (after the animals had become accustomed to the cages and the diet), during which the urine and fæces were collected from

each pair of rats every second day; a preliminary control period of 10–14 days was followed by a period of 12–14 days in which the extract was

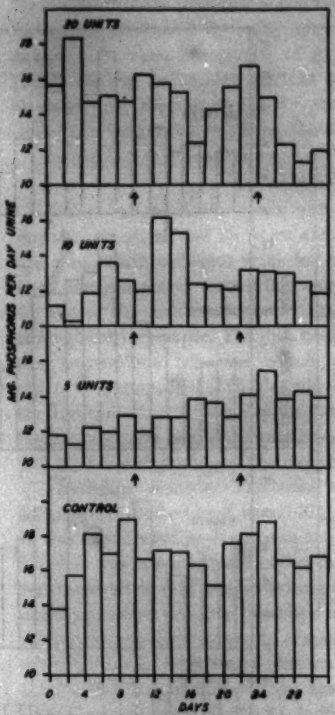

Fig. 3.

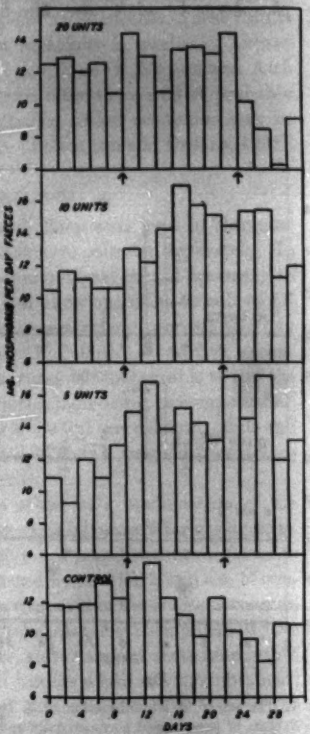

Fig. 4.

Fig. 3. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of phosphorus in the urine of adult rats.

Fig. 4. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of phosphorus in the faeces of adult rats.

injected subcutaneously in daily doses of 5, 10, or 20 units, and a recovery period. The results are shown in Figs. 1, 2, 3, and 4. Each curve gives the excretion per rat per day in mg. and each represents the

average excretion of three pairs of rats; three pairs of untreated controls were also followed. The most striking effects are seen in the increase in urinary calcium, which on the largest dose attains some fifty times the control value. It will be observed that the effect varies with the dose, and that it is not maintained throughout the period of treatment (the beginning and end of which are shown in the figures by arrows) but attains a peak on the fourth day and thereafter rapidly declines. In fact the rats appear to become immune rapidly or refractory to the extract, a phenomenon which has also been observed in dogs and in human subjects, and which is the object of further study in the later experiments. The calcium excretion in the fæces is also increased, but to a much less striking extent, in the period of treatment. Since the total "extra calcium" excreted, even on the largest dose, is not over 70 mg., it is not surprising that Day [1930] was unable to obtain significant reductions in the total body calcium of rats receiving much smaller doses of the hormone. The excretion of phosphorus in the urine is somewhat increased during the period of treatment, but the relative change is not nearly as great as that in calcium excretion, nor is there any evidence that it occurs more rapidly; the fæcal phosphorus does not appear to be significantly affected. None of these rats showed any ill-effects from the series of injections except a slight loss of weight; the administration of 40 units daily, however, was found to produce cachexia in 3 days and death in 5 or 6; the observation of Tweedy and Chandler [1929], that the congestion and hyperæmia of the stomach characteristic of dogs receiving excessive doses of parathyroid extract does not appear in rats, was confirmed by post-mortem examinations.

The effect of parathyroid hormone upon the serum calcium of adult rats. Blood was collected from rats by severing the carotid artery and jugular vein on one side and allowing the animals to bleed to death into a centrifuge tube; in order to obtain sufficient serum for the determination of calcium by the technique of Clark and Collip [1925], it was necessary to pool the blood from two rats. The average serum calcium of 40 pairs of rats on the diet described was found to be 10·3 mg. p.c., with a standard deviation of only 0·22 mg. p.c. Rats receiving 10, 20, or 40 units of parathyroid hormone daily were killed at various intervals and the serum calcium values obtained are plotted in Fig. 5, in which each point represents the average of determinations made on from three to eight pairs of rats. It is clear that marked hypercalcæmia can be produced, that it is to some extent proportional to the dose injected, and that it is not maintained throughout treatment but tends to return towards normal levels.

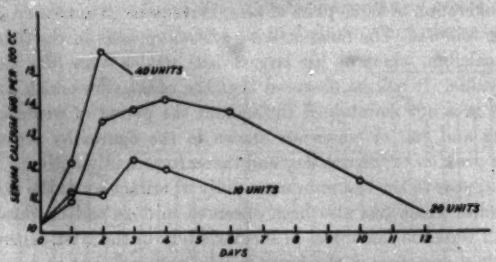

Fig. 5. The effect of administration of 10, 20 and 40 units of parathyroid hormone daily upon the serum calcium of adult rats.

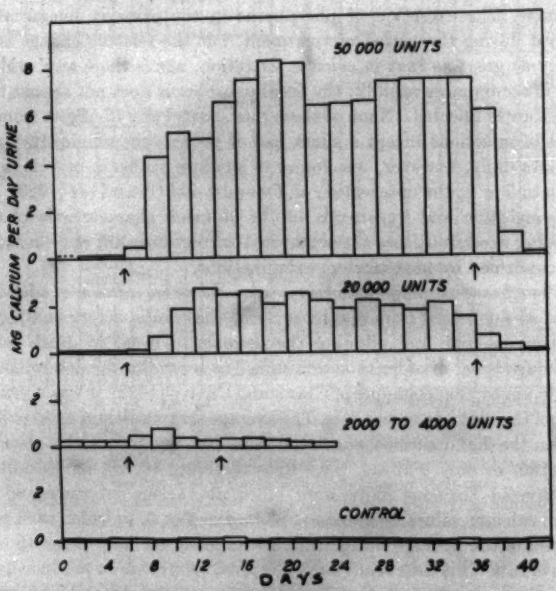

Fig. 6. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of calcium in the urine of adult rats.

The effect of irradiated ergosterol upon the excretion of calcium and phosphorus by adult rats. This experiment was carried out in the same manner as the first experiment. The irradiated ergosterol used was a sample prepared by Mead, Johnson and Co. for research purposes and was stated to have the potency 10,000 $X \pm 10$ p.c., assayed by the

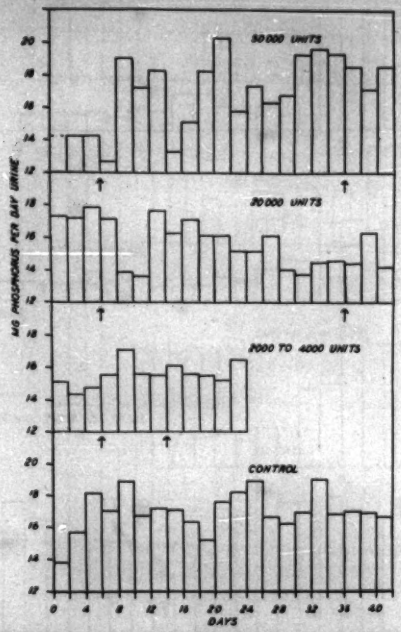

Fig. 7. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of phosphorus in the urine of adult rats.

method of Bills, Honeywell, Wirick and Nussmeier [1931]; according to the figures given by these workers, it therefore contained one million international rat units per c.c. Various dilutions in olive oil were prepared and were administered orally by means of a pipette in doses of 2000, 4000, 20,000 and 50,000 units daily, in each case in a volume of 0·1 c.c. per dose. The results of the experiments are shown in Figs. 6, 7, 8 and 9; as they are on the whole in harmony with those of other workers,

they do not require extended discussion. With the larger doses, there is a great increase in the excretion of calcium in the urine with no regular change in urinary phosphorus, and, in contrast to the experiments with

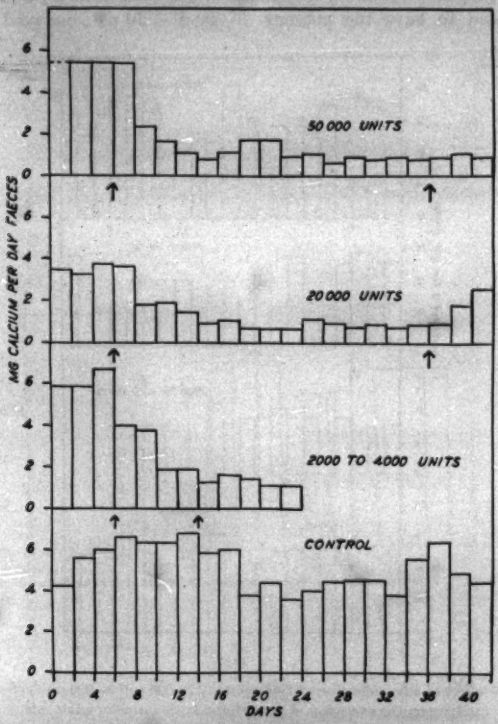

Fig. 8. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of calcium in the fæces of adult rats.

parathyroid extract, a very distinct decrease in the fæcal excretion of both elements. Except on the highest doses, the rats did not appear to suffer any ill effects from the treatment. These results are not readily brought into line with the theory that the chief effect of large doses of irradiated ergosterol is to stimulate the parathyroid glands.

The administration of irradiated ergosterol to rats refractory to parathyroid hormone. In this experiment a study was made of the calcium and phosphorus excretion of a group of rats which received first 10 units of parathyroid hormone daily for 12 days on two separate occasions, then

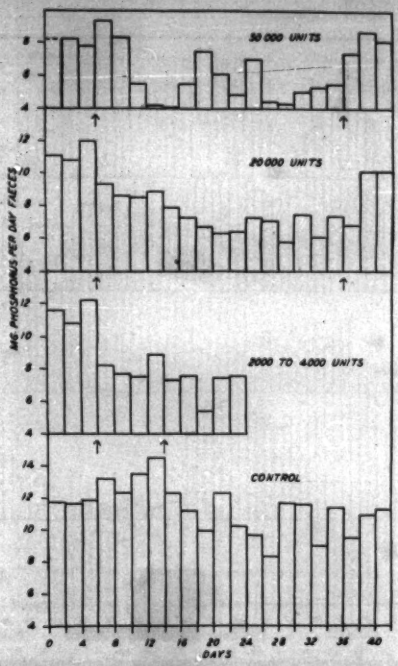

Fig. 9. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of phosphorus in the faeces of adult rats.

received 50,000 units of irradiated ergosterol daily for 30 days, and lastly received 10 units of parathyroid hormone daily for 14 days. The results are shown in Figs. 10 and 11, in which the parathyroid periods are marked by a shaded block and the vitamin period by a black block. The urinary calcium increased in the usual way at the beginning of the first parathyroid period, but showed almost no response in the second and third parathyroid periods, though it increased as usual, with the usual

concomitant decrease in fæcal calcium, in the intervening vitamin period. This experiment shows firstly, that rats which have once become immune or refractory to parathyroid hormone do not readily regain sensitivity, and secondly, that such rats respond as usual to the treatment with irradiated ergosterol.

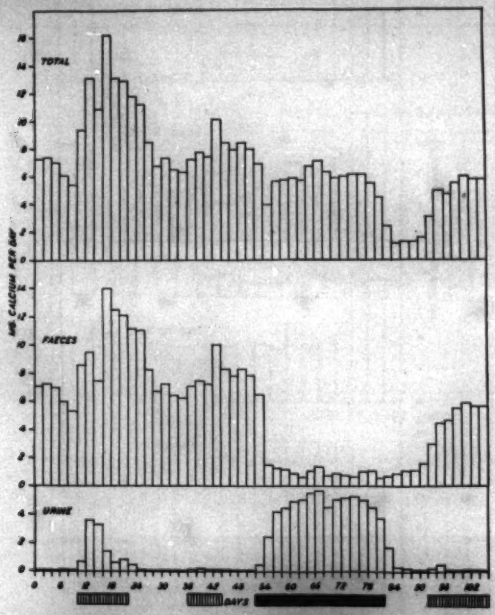

Fig. 10. Comparison of the effect of successive administration of parathyroid hormone with that of irradiated ergosterol upon the excretion of calcium of adult rats:

10 units of parathyroid hormone daily.

50,000 units of vitamin D daily.

The effect of urine of immune rats receiving parathyroid hormone on the calcium excretion of responsive rats. Since it was felt that the animals which had developed "immunity" to parathyroid hormone might be excreting the injected hormone in the urine, an investigation of the point was undertaken. Five pairs of rats which had previously received 10 units of parathyroid hormone daily in three periods, and which no longer

showed any response to the injections, were treated with 20 units of parathyroid hormone daily. One pair ("*A*") was kept as a control, the urine and fæces being analysed for calcium, which did not increase during the treatment. The urine of the other four pairs was injected into

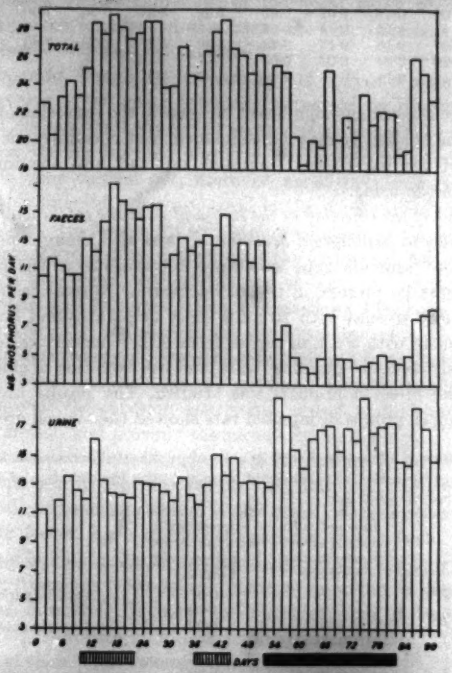

Fig. 11. Comparison of the successive administration of parathyroid hormone with that of irradiated ergosterol upon the excretion of phosphorus of adult rats.

three pairs of normal rats (*X*, *Y* and *Z*) whose calcium excretion was followed. The results are summarized in Table I. It is seen that though the amount of urine injected into each of the normal (and presumably responsive) rats daily might have contained 26·6 units of parathyroid hormone, if quantitative excretion occurred, and though the urine was collected in the presence of acetic acid and chloroform to prevent destruction of any hormone present, yet none of the rats receiving injections

TABLE I. Effect of administration of urine of rats immune to the action of parathyroid hormone, upon the excretion of calcium of normal adult rats expressed in mg. per day.

	Urine				Fæces				
Date 1932	A Control	X	Y	Z	A Control	X	Y	Z	
Feb. 22	0·07	0·18	0·02	0·02	3·30	5·67	5·50	7·00	Control
„ 23	0·11	0·04	0·13	0·02	—	—	—	—	Experimental
„ 24	0·07	0·15	0·17	0·23	3·30	4·75	5·70	7·40	period
„ 25	0·15	0·04	0·04	0·04	—	—	—	—	
„ 26	0·12	0·14	0·08	0·29	4·90	5·37	5·37	7·70	

of this urine showed any significant increase in the excretion of calcium in the urine or faeces. It may be concluded that certainly not more than one-fifth of the parathyroid hormone injected into the immune rats appeared in the urine.

The effect of the injection of the serum of immune rats upon the response of normal rats to parathyroid hormone. It was also thought possible that in "immune" animals some substance antagonistic to the parathyroid extract might be present in the blood serum. Normal responsive rats were therefore treated with 20 units daily of parathyroid hormone and simultaneously with 2 c.c. of serum from either normal, responsive rats or previously treated "immune" rats, and the excretion of calcium in the urine of the injected animals was studied. The results are shown in Table II. Both groups of injected rats showed the typical great increase

TABLE II. Effect of administration of 20 units of parathyroid hormone and serum of rats immune to the action of parathyroid hormone, upon the excretion of calcium in the urine of normal adult rats.

Date 1932	Injected with normal serum + parathyroid hormone	Injected with immune serum + parathyroid hormone	
Jan. 3	0·15	0·07	Control period
„ 5	0·18	0·09	
„ 7	1·70	0·33	Experimental period
„ 9	4·80	5·93	
„ 11	2·44	1·25	
„ 13	0·39	0·39	
„ 15	0·30	0·38	

in urinary calcium during the first part of the period of parathyroid treatment, and neither normal serum nor "immune" serum inhibited this effect.

IV. DISCUSSION.

Although the rat is relatively refractory to parathyroid hormone, when compared with the dog or the man, nevertheless it is possible to produce an increased serum calcium and an increased excretion of

calcium in the urine and fæces by the injection of moderate doses. Of these effects, the greatest relatively is that upon the urine calcium in the first few days of treatment. Since this effect is obtainable with doses as small as 5 units daily per rat, and is roughly proportional to the dose, it suggests that a valuable method of biological assay of parathyroid extracts might be founded on this basis, the only objection being the tedious nature of the analysis required[1].

The apparent immunity to parathyroid extracts which the rats rapidly develop is of great interest. Attempts to demonstrate the presence of an immune body in the serum, or an increased permeability of the kidney to the injected hormone, completely failed. Since these experiments were carried out, however, an entirely new light on the nature of this immunity has appeared in the work of Selye [1932], in this laboratory. He has shown by histological study of the bones of rats receiving parathyroid extract, that while the first response is a formation of numerous osteoclasts with rarefaction of bone (doubtless leading to hypercalcæmia and increased calcium excretion), this is succeeded by the disappearance of the osteoclasts, the formation of numerous osteoblasts, with bone apposition proceeding to such an extent that the picture of "marble-bone disease" can be produced experimentally. In this second phase of the reaction one would expect to find a decrease in calcium excretion and possibly subnormal serum calcium.

The metabolic effects of large doses of vitamin D (irradiated ergosterol) are quite unlike those of parathyroid hormone. There is once more an increased excretion of calcium in the urine, but it is accompanied, as others have found, by a decreased excretion in the fæces; moreover, animals may be responsive to irradiated ergosterol when they are almost completely "immune" (as far as calcium excretion is concerned) to parathyroid hormone. It may be concluded that if large doses of irradiated ergosterol do stimulate parathyroid secretion, this is but a small part of their pharmacological action.

V. Summary.

1. General and chemical methods for following the excretion of calcium and phosphorus in the urine and fæces of rats are described.

2. The injection of parathyroid hormone, in doses of 5 to 20 units daily, leads to an increase in serum calcium, an increase in fæcal calcium

[1] After this article had gone to press F. J. Dyer reported before the Physiological Society, London, *J. Physiol.* 75, 13 *P*, 1932, a method for the estimation of parathyroid hormone based on the rise in urinary calcium of male rats.

and urine phosphorus, and a relatively more striking increase in urine calcium, which however is not long maintained. Experiments on the nature of the apparent immunity to the hormone developed under treatment are described and discussed.

3. Oral administration of irradiated ergosterol, in doses of 20,000 to 50,000 units daily, leads to an increase in urine calcium but a decrease in fæcal calcium and phosphorus. This effect is also obtainable in rats which have become "immune" to parathyroid hormone.

I wish to express my thanks to Prof. J. B. Collip and to Dr D. L. Thomson for help and advice during the course of this work.

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INORGANIC SULPHATE EXCRETION BY THE HUMAN KIDNEY.

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In putting forward his modern theory of renal function, Cushny [1917] pointed out that if any substance could be found which was eliminated entirely by glomerular filtration and was neither reabsorbed nor secreted by the cells of the renal tubules, then that substance could be employed to determine the actual rate of glomerular filtration. The truth of this can scarcely be questioned unless we suppose that the process occurring in the glomeruli is not one of simple filtration.

For thus estimating glomerular filtration rate, Cushny originally suggested that urea fulfilled the requirements, but later, in view of the accumulating evidence against an uncomplicated filtration of urea, abandoned that substance in favour of inorganic sulphate [1926].

Mayrs [1922] and White [1923] had already employed sulphate excretion for the same purpose in animals. In 1926 Rehberg pointed out the apparent relative advantages of creatinine excretion as an index of filtration rate, and in more recent papers has published observations which he interprets as supporting the view that creatinine fulfils the necessary requirements, *i.e.* is filtered off in the glomerular filtrate in the same concentration as in the plasma, and is neither reabsorbed nor secreted by the tubule cells. For any substance fulfilling these conditions the mode of calculation of the filtration rate is the same.

Thus if X_p and X_u be the concentrations of the substance in plasma and in urine respectively, expressed as mg. per 100 c.c., and V be the volume of urine excreted in c.c. per unit of time, then the glomerular filtration rate will be $100\frac{X_u \cdot V}{X_p}$ c.c. in the same unit of time. This ratio, determined for creatinine, Rehberg calls the "glomerular filtration rate," but in the present paper it is considered preferable to employ the ratio $\frac{X_u \cdot V}{X_p}$ and to call this the "clearance for substance X in keeping with the nomenclature now in frequent use in discussing urea excretion.

The terms "clearance for substance X" and "glomerular filtration rate as estimated by substance X" thus refer to essentially the same ratio, but differ in that the former attempts no interpretation of the physiological significance of the ratio, whilst the latter ascribes to it a meaning which as yet is not strictly justified. For this reason the former term is considered preferable.

Of the various substances occurring naturally in the urine, two, viz. creatinine and inorganic sulphate, have been held to afford reliable indices of the extent of glomerular filtration. If the claims for both substances are to be justified it must be shown that the results obtained by the use of creatinine are the same as those obtained with sulphate, and that this agreement holds under all conditions both physiological and pathological. In other words, the clearance for sulphate must always be the same as that for creatinine.

Accordingly Mayrs [1922] and White [1923] showed that when the blood sulphate concentration was raised to abnormally high levels by intravenous injection of sodium sulphate, the concentration ratios for creatinine and for sulphate were practically the same. Earlier technical difficulties in the accurate estimation of inorganic sulphate in the low concentrations, encountered in the plasma of normal animals and of men, prevented these observations from being extended to include the normal subject.

Nevertheless Poulsson [1930] sought to show that the two clearances were the same in man. He assumed the concentration of plasma inorganic sulphate in his experiments to lie within the limits of normal given by Wakefield [1929]. These limits were considerably below those obtained by other workers using different methods, and more recently Wakefield himself, in collaboration with Power and Keith [1931], and using an improved method, has obtained higher normal values in good agreement with those of other investigators. The deductions of Poulsson are thus based on figures which Wakefield himself has discarded as too low. It is clear therefore, that Poulsson's suggestion that the creatinine and sulphate clearances are of the same magnitude in man cannot be accepted on the evidence he provides.

In the present paper results of a number of direct determinations of the sulphate clearance in normal and in nephritic men are presented in which this claim of Poulsson could not be confirmed. The theoretical bearing of this fact is also considered.

Methods.

Creatinine in urine was determined by the usual method of Folin [1914].

Creatinine in plasma was determined colorimetrically in a Folin-Wu filtrate. Details of the precautions employed have been given elsewhere [Cope, 1931 *a*].

Sulphate in urine. The gravimetric method of Folin [1905] was found entirely satisfactory and was used throughout.

Sulphate in plasma and serum. This has been estimated by two entirely independent methods: (i) Nephelometrically, by a modified Denis technique giving an accuracy of about 10 p.c. [Cope, 1931 *b*]. (ii) By benzidine precipitation and microtitration [Cope, 1931 *b*], a method in which the error does not exceed 5 p.c. in the concentrations estimated.

In the tables of results these methods are designated by the letters N and B respectively.

Urea in blood and in urine was determined by the urease and aeration methods of Van Slyke and Cullen [1914].

Results.

Table I shows the results of a series of inorganic sulphate clearance determinations made on healthy young adults, in which the nephelometric method of serum sulphate estimation was used. Observations were made during the morning hours on subjects who had had no breakfast and who were during the observational period moving about the laboratories. The condition was, therefore, not one of complete rest, nor was a diuresis provoked. Urine was collected over a period of one hour, in the middle of which a sample of venous blood was taken for sulphate analysis in the serum.

The results are divided into two groups. In the first, plasma sulphate concentration was normal; in the second it had been raised somewhat by the oral ingestion of 10 g. of sodium sulphate the previous evening. It will be seen that, with one exception, the sulphate clearances lie between 26·0 and 51·3, with a mean value for each group of about 35·5.

Although, unfortunately, simultaneous creatinine clearance determinations were not made in this series, there can be little doubt that these would be considerably higher than 35·5. Holten and Rehberg [1931] give a minimum normal value of about 60 with a mean of about 90.

TABLE I. Sulphate clearances in normal human subjects. (Nephelometric method.)

A. Normal plasma sulphate concentration.

Subject	Volume of urine per hour	Urine inorganic sulphate mg. S per 100 c.c.	Inorganic sulphate excretion mg. S per hour	Serum inorganic sulphate	Sulphate clearance (hourly)
Hunt	39·5	99·00	39·00	1·11	35·4
Osborn	51·5	50·86	26·20	0·72	36·1
Osborn	86·0	46·50	40·00	0·78	51·3
Cope	39·0	61·60	24·00	0·80	30·0
Palin	47·5	107·00	50·88	1·15	44·2
Cope	41·2	55·25	22·76	1·17	19·5
Starling	18·5	284·80	52·68	1·60	32·9
				Mean 1·05	35·6

B. 15–18 hours after oral ingestion of sodium sulphate (10 g.).

Osborn	57·0	118·00	67·64	1·76	38·2
Talbot	61·0	115·30	70·30	1·76	40·0
Cope	54·0	160·60	86·90	1·87	46·6
Watson	27·0	249·20	67·05	1·92	34·9
Palin	38·0	156·70	59·57	2·24	26·6
Bosworth	189·0	39·48	74·63	2·26	33·0
Cope	46·0	133·60	61·45	2·27	27·0
Swan	66·1	134·10	88·52	2·42	36·3
				Mean 2·06	35·3

Poulsson [1930] obtained creatinine clearance values from 80 to 108 per hour, and the present writer has also obtained similar figures.

A series in which simultaneous determinations of urea, creatinine and sulphate clearances were all made on the same subject is shown in Table IV. With the exception of (10 *a*) and (11 *a*), these determinations were all made with the subject at rest, and in the presence of a large diuresis provoked by combined water and urea ingestion, *i.e.* the so-called Addis conditions.

That such conditions do not materially influence the sulphate clearance is indicated by experiments (10 *a*) and (11 *a*) which were performed on the same mornings as (10) and (11) respectively, but during moderate exercise and in the absence of diuresis. Here a very considerable change in conditions has had no significant effect on the sulphate clearance. In this series the sulphate clearance, far from equalling the creatinine clearance, seldom exceeds one-third of the value of the latter, and tends, indeed, to be slightly lower than the maximum urea clearance. That the same essential ratio between the three clearances tends to persist when the functional activity of the kidney is reduced by disease, is shown in the observations on nephritic human subjects given in Table III.

TABLE II. Comparison of urea, sulphate and creatinine clearances in human subject C. L. C.

No.	Creatinine clearance: Uncorrected	Creatinine clearance: Corrected	Urea clearance (hourly)	Volume urine per hour	Urine inorganic sulphate mg. S per 100 c.c.	Excretion inorganic sulphate mg. S per hour	Plasma sulphate mg. S per 100 c.c.	Inorganic sulphate clearance (hourly)	Method
1	59·3	90·8	—	662	6·21	41·1	1·34	30·9	N
2	66·9	113·3	—	656	5·17	33·9	0·97	34·8	N
3	101·0	121·0	42·2	478	7·31	34·9	0·90	38·8	N
4	120·2	135·1	45·0	564	6·48	36·6	1·14	32·1	N
5	119·4	137·7	42·1	442	7·87	34·8	1·34	25·9	N
6	100·5	120·8	36·0	438	7·48	32·8	1·78	18·4	N
7	100·0	115·0	35·0	734	3·98	29·3	1·11	26·4	B
8	109·2	125·0	—	490	8·28	40·56	1·18	34·4	B
9	104·6	117·5	—	820	4·35	35·64	1·00	35·6	B
10	110·0	125·0	—	667	4·96	33·08	0·905	36·5	B
10 a	115·4	126·0	—	83	40·1	33·23	0·88	37·8	B
11	107·0	113·0	—	748	4·24	31·7	1·01	31·4	B
11 a	97·8	109·5	—	40·6	94·4	38·3	1·11	34·6	B
Mean	—	119·2	40·0	—	—	—	1·13	32·1	—

Note. The letters N and B in the last column indicate respectively the nephelometric and benzidine methods of estimating plasma sulphate.

Corrected creatinine clearances are obtained by subtracting 0·5 mg. from the estimated plasma creatinine value and recalculating the ratio [see Cope, 1931 *a*].

TABLE III. Comparison of sulphate clearances with urea and creatinine clearances in nephritis.

No.	Maximum urea clearance	Uncorrected creatinine clearance	Corrected creatinine clearance	Volume of urine per hour	Plasma inorganic sulphate mg. S per 100 c.c.	Urinary sulphate mg. S per 100 c.c.	Excretion inorganic sulphate mg. S per hour	Sulphate clearance (hourly)	Method
1	21·9	59·5	67·3	105	0·525	13·3	14·0	26·6	B
2	35·6	95·0	110·0	670	1·87	8·99	41·5	22·2	N
3	—	69·9	78·2	471	3·14	13·96	65·8	20·9	N
4	27·1	29·0	52·7	424	2·90	14·15	60·0	20·7	N
5	20·0	59·9	65·4	124	2·44	40·3	50·0	20·5	B
6	—	56·8	61·7	296	1·67	11·12	32·6	19·6	B
7	—	55·2	60·1	114	2·47	40·3	45·9	18·6	B
8	—	74·2	81·3	166	0·99	10·0	16·7	16·8	B
9	22·0	39·6	52·3	184	1·90	15·4	28·3	14·9	N
10	17·2	41·2	45·4	190	2·44	19·06	36·2	14·8	N
11	—	78·5	88·8	148	0·82	8·22	12·2	14·8	B
12	21·3	54·9	62·4	128	1·04	10·22	13·1	12·6	B
13	4·47	11·9	12·85	50	5·80	93·1	46·5	8·0	N
14	6·53	14·3	17·7	61	5·00	53·7	32·6	6·5	N
15	4·07	7·6	8·14	61	8·00	52·2	32·0	4·0	B
16	—	—	—	25	16·00	151·8	37·9	2·27	N

In the final column the letters N and B indicate respectively the nephelometric and benzidine methods of estimating plasma inorganic sulphate.

Discussion.

The important point which it is desired to stress is that the value of the sulphate clearance in the human subject, healthy or nephritic, is consistently below that for the creatinine clearance, and is usually only about 30 p.c. of the latter. In no case has the sulphate clearance even approached the value for the creatinine clearance, and this remains true even if uncorrected creatinine clearances are considered.

It is evident therefore, that both substances cannot be regarded as indices of glomerular filtration rate.

Several alternative explanations of the difference are possible. If it be supposed that the creatinine clearance does indeed represent the glomerular filtration rate, then we must conclude either that about two-thirds of the plasma inorganic sulphate exists in the circulating blood in a non-diffusible form, or else that a similar fraction is reabsorbed in the tubules. The former possibility is unlikely since blood may be completely freed of its inorganic sulphate by dialysis through collodion. It must be conceded, however, that as this process takes some considerable time, the possibility that an indiffusible compound is gradually converted into a diffusible form during dialysis cannot be definitely excluded.

The assumption that two-thirds of the sulphate filtered in the glomeruli is reabsorbed during its passage down the tubules, also presents difficulties, for if it were so, it would be reasonable to suppose that the induction of a heavy diuresis, by affording less opportunity for reabsorption, would lead to an increase in the sulphate excretion rate and so in the sulphate clearance. Such an increase apparently does not occur.

Moreover, the fact reported by Mayrs [1922] and by others, that ureteric obstruction reduces urea excretion much more than it does that of sulphate, has been interpreted by holders of the filtration-reabsorption view as evidence that urea is more readily reabsorbed than is sulphate. If this conclusion be true, then the sulphate clearance should be higher than that for urea. For although maximal urea clearances are being considered, the reabsorption theory must suppose that even from such dilute urines 50 p.c. or more of the filtered urea is reabsorbed, in order to account for the fact that the creatinine clearance is so much higher than the urea clearance.

Yet in the instances here reported, the sulphate clearance tends rather to be lower than the urea clearance, and the experience of Sager [1930] in dogs would appear to be similar, for his results show sulphate clearances sometimes slightly below, and sometimes rather above the urea

clearances. Furthermore, if we postulate such a large reabsorption of sulphate then it is necessary to suppose that through the tubule cells sulphate escapes with ease but creatinine with extreme difficulty, although into other tissues of the body creatinine is readily diffusible whilst sulphate only passes with great difficulty [Denis and Leche, 1925].

If, however, the possibility of secretion into the lumen through the tubule cells be conceded, then the explanation becomes relatively simpler. We may now suppose that the actual glomerular filtration rate is equal to, or somewhat less than, the value indicated by the average sulphate clearance, *i.e.* about 3·5 litres per hour in the normal human adult. Let us assume that the actual glomerular filtration be only 3 litres per hour. This will correspond to an hourly glomerular clearance activity of 30 for both creatinine and sulphate. If, further, we assume that the sulphate from 500 c.c. of plasma and the creatinine from 9 litres of plasma are eliminated through the tubules, then the observed values can be accounted for, for these figures correspond to tubular clearing activities of 5 and 90 respectively, bringing the total clearing activity of glomeruli and tubules combined, *i.e.* of the kidneys as a whole, up to 35 and 120 respectively. Such a view requires a behaviour of sulphate and of creatinine towards the tubule cells which is similar to that shown towards other tissues, and consequently would appear preferable to the filtration-reabsorption view which necessitates relative rates of passage of these substances through the tubule cells which are the reverse of those through other tissues of the body.

Rehberg bases his hypothesis that creatinine is filtered off, but neither reabsorbed nor secreted in the tubules, upon the following points [Holten and Rehberg, 1931].

(i) Its concentration ratio is higher than that of any other substance.

(ii) The excretion rate is independent of the volume of diuresis.

(iii) Change in osmotic pressure of the plasma proteins is associated with a change in rate of creatinine excretion in the same direction as would be anticipated if the excretory process were one of ultrafiltration in the glomeruli.

Of these, the high concentration ratio and the constancy of excretion with changing urine volume are equally compatible with a secretory mechanism. The relation to plasma protein osmotic pressure, whilst affording evidence suggestive that some creatinine is filtered off in the glomeruli, cannot be taken as evidence that all the creatinine is excreted in this manner.

In a recent study of the action of cyanide on the isolated kidney, Bayliss and Lundsgaard [1932] observed a very marked drop in the creatinine clearance. Being unwilling to believe that the glomerular filtration rate really fell to such an extent under the influence of cyanide alone, these writers postulated a progressively increasing leakage of fluid and of creatinine outwards from the tubule lumen. Their results, however, are equally compatible with the view that cyanide inhibits a creatinine secretory mechanism in the tubules, so that at the end of the experiment only that fraction of the creatinine which is filtered off in the glomeruli appears in the urine. Whether simultaneously any reduction in the true filtration rate did occur in their experiments can scarcely be decided, but it would seem probable.

More direct evidence pointing to a secretory elimination of creatinine is contained in the experiments of Edwards and Condorelli [1928] on the aglomerular kidneys of certain fishes. Nor can the similarity of behaviour of phenol red and creatinine during excretion which was shown by Marshall and Kolls [1919] be brought forward as suggesting a filtration, for although Oliver and Shevky [1929] present evidence that phenol red is eliminated solely by glomerular filtration, yet Höber [1930] maintains that it is secreted by the tubules, and the more recent work of Chambers on the behaviour of tissue cultures of embryonic tubules suggests strongly a secretory mechanism for the dye.

As regards the mechanism of excretion of inorganic sulphate, the situation is scarcely more clear. White [1923], from his experiments on the concentration ratios, concluded that sulphate comes in part through the glomeruli and in part through the tubules by secretion. Starling and Verney [1924] interpreted the fall in rate of excretion and in concentration of sulphate in the urine from a kidney poisoned by cyanide, as evidence of an active secretory process. Such an interpretation was, however, based on the belief that cyanide had no effect on the volume of glomerular filtrate, an assumption which must be considered doubtful in view of the work of Höber and Mackuth [1927] and of Bayliss and Lundsgaard [1932].

Of the attempts to dissociate glomerular from tubular elimination of sulphate in the frog, the earlier ones of Cullis [1906] and of Atkinson, Clark and Menzies [1921] must be interpreted with caution in view of the possibility of access of the tubular perfusion fluid to the vessels of the glomerular system. In the experiments of Yoshida [1924], this danger would appear to have been lessened. Yoshida found that when sulphate was supplied to the glomeruli of the frog's kidney it was well excreted and

concentrated, but that when applied to the tubules alone it appeared in the urine only in traces. More recently again, Kawasoe [1930], also working on frogs, has stated his conclusions that sulphate is excreted mainly by the glomeruli and possibly also slightly by the tubules. He also believed that no significant reabsorption of sulphate occurred.

Such results as these would thus agree well with the view presented here that the glomerular filtration rate is equal to, or somewhat less than the figure indicated by the sulphate clearance.

It seems to the writer highly desirable that this possibility should not be lost sight of. There is, strictly speaking, at present no real justification for supposing that the creatinine clearance is a more reliable index of glomerular filtration than is the sulphate clearance. There has of more recent years been a tendency to forget that Rehberg's suggestion was admittedly no more than a hypothesis based on an assumption concerning the behaviour of creatinine in the kidney. The rival claims of sulphate as a filtration index would seem to be at least equally strong. Possibly neither is a true index.

Whilst this work was being prepared for the press, there appeared a report of similar work arising from the same line of thought by Hayman and Johnston [1932]. Using a different method of estimating plasma sulphate, they obtain essentially the same results. They find the concentration ratio for inorganic sulphate much below that for creatinine, and in most cases also below that for urea. With such results those presented here are in complete agreement. These workers interpret their findings as evidence of back diffusion in the tubules, a conclusion which, as pointed out above, the present writer considers the less likely possibility.

Summary.

The excretory activity for inorganic sulphate of the human kidney, either healthy or nephritic, is only about one-third of that for creatinine.

Calculations of the glomerular filtration rate based on the excretion of inorganic sulphate thus give a value only about one-third of that indicated by the excretion of creatinine.

The theoretical consequences of this fact are considered.

This work was done under the tenure of a Beit Memorial Fellowship for Medical Research, and constitutes part of that submitted in a Thesis for the degree of Doctor of Medicine in the University of Oxford in 1931.

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577.174.5:612.133

A STUDY OF THE INFLUENCE OF ADRENALINE ON THE SYSTEMIC BLOOD FLOW.

By HENRY BARCROFT,

Harmsworth Scholar, St Mary's Hospital, London.

(*From the Physiological Laboratory, Cambridge.*)

The object of this paper is twofold. Firstly to describe the changes in the systemic flow in dogs after the injection of adrenaline. Secondly to demonstrate certain phenomena which adrenaline brings about.

The systemic output has been measured with a mechanical stromuhr. This instrument has allowed a more complete study of the systemic flow under the influence of adrenaline than has been hitherto undertaken. The instrument and the accuracy of its readings have been fully described in a previous paper [Barcroft, 1929].

I. *A description of the changes in the systemic flow observed after adrenaline injection.*

Ten separate experiments have been performed. Each dog weighed approximately 10 kg. The operative procedure necessary for diverting the systemic flow through the mechanical stromuhr is fully described elsewhere [Barcroft, 1931 *a*]. The preparation is shown diagrammatically in Fig. 1 *a*. The position of the stromuhr and of the injections of adrenaline are indicated. The injections were made either intravenously or into the aorta peripheral to the coronary arteries but central to all other branches. Each injection contained from 0·05 to 0·1 mg. of adrenaline. The action of adrenaline on the vascular system was studied after section of the vagi. In some experiments the brain was destroyed by temporary obstruction of its blood supply; this did not affect the variations in the systemic flow seen after adrenaline.

Figs. 2, 3, 4 and 5 show variations in the systemic flow, arterial blood-pressure and heart rate in response to adrenaline. They illustrate the following:

(1) Figs. 2 and 3 are drawn from data supplied by one experiment. They show that whether the adrenaline was injected intravenously or

into the aorta the systemic flow varied, but was not much increased or decreased.

(2) Figs. 4 and 5 are drawn from data supplied by another experiment. They show that whether the adrenaline was injected intravenously or into the aorta the systemic flow varied and was greatly increased.

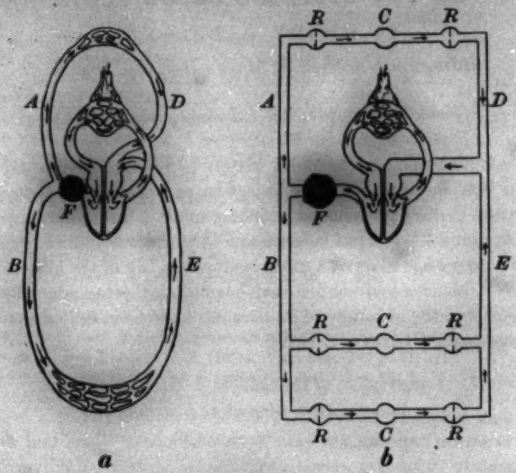

Fig. 1.

a. Diagram of animal's vascular system: *A*, brachiocephalic artery; *B*, thoracic aorta; *D*, superior vena cava; *E*, inferior vena cava; *F* represents the position of the stromuhr; *F* represents the position of the aortic injections of adrenaline; *F* represents the point at which the screw clamp was applied; *E* represents the position of the injections of adrenaline into the femoral vein.

b. Diagram of the cardio-pulmonary system attached to the artificial peripheral vascular system: *A*, tube representing the brachiocephalic artery; *B*, tube representing the thoracic aorta; *D*, tube representing the superior vena cava; *E*, tube representing the inferior vena cava; *R*, resistance; *C*, extensible part of peripheral vascular system; *F* represents the position of the stromuhr.

In most experiments adrenaline did not greatly increase the output, but all grades between the two extremes illustrated have been observed. In Figs. 4 and 5 the initial blood-pressure is seen to have been very low, but a large increase in the systemic flow has been seen in animals with a normal blood-pressure.

The curves also show:

(3) The systemic flow is at first increased by intravenous injections. This increase is coincident with a rise in the heart rate and in the arterial blood-pressure (Figs. 2, 4).

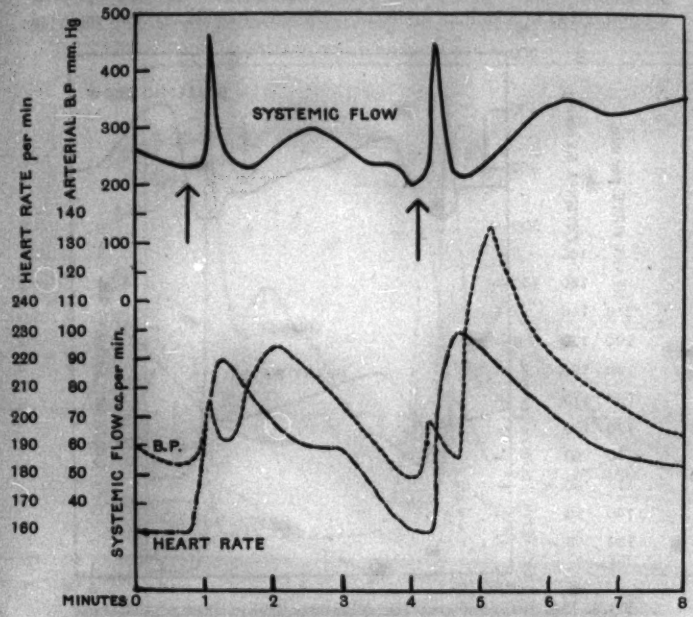

Fig. 2. Dog. Brain destroyed. The arrows mark times at which intravenous injections of adrenaline were made.

(4) The systemic flow is at first decreased by the aortic injections. This decrease accompanies the initial rise in the arterial blood-pressure.

The above effects were found in all typical experiments.

Further experiments of a different nature have provided some explanation for the above changes in the systemic flow.

II. *The influence of adrenaline on the cardio-pulmonary system causing increase in systemic flow.*

To observe how the action of adrenaline upon the cardio-pulmonary system affected the systemic flow, the masking effect of its action on the peripheral vascular system had to be eliminated. This was done by using a preparation in which an artificial peripheral vascular system replaced

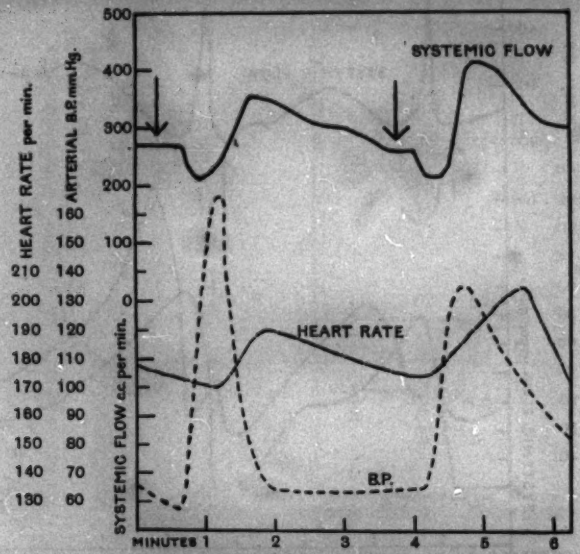

Fig. 3. Dog. Brain destroyed. The arrows mark times at which adrenaline was injected into the aorta.

the animal's peripheral vascular system. The preparation is fully described in a previous paper [Barcroft, 1931 *b*] and is diagrammatically shown in Fig. 1 *b*.

The following typical result has been obtained. Fig. 6 I shows that the flow round the artificial peripheral vascular system was greatly increased by adrenaline.

The conclusion drawn from this experiment is that, in the animal, the action of adrenaline on the cardio-pulmonary system tends to increase the systemic flow in qualitatively the same manner as shown in Fig. 6 I.

III. *The influence of adrenaline upon the peripheral resistance causing decrease in systemic flow.*

The fact that adrenaline causes increase in the arteriole tone and in the peripheral resistance is well known.

To imitate this action a screw clamp was placed in position round the animal's aorta at the point *F* shown in Fig. 1 *a*. Increase in the peripheral

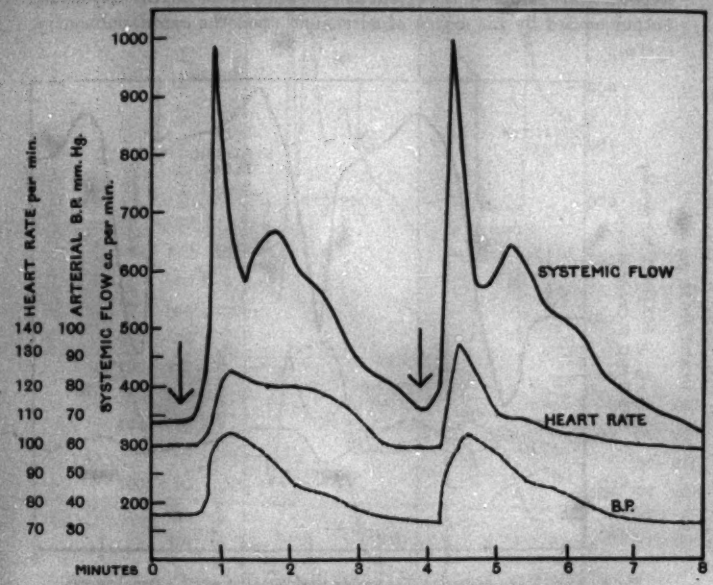

Fig. 4. Dog. Brain destroyed. The arrows mark times at which intravenous injections of adrenaline were made.

resistance was effected by partially tightening the screw, thereby partially occluding the aorta. Typically increase in the peripheral resistance always decreased the systemic flow. Fig. 6 II shows that in one instance increase in the peripheral resistance caused the arterial blood-pressure to rise from 140 to 180 mm. of mercury. The systemic flow dropped from 800 c.c. to 580 c.c. per minute and remained constant till the occlusion was removed 40 seconds later.

The conclusion drawn from this experiment is that, in the animal, the action of adrenaline causing increase in the peripheral resistance tends to decrease the systemic flow.

IV. *Explanation of variations in the animal's systemic flow due to adrenaline.*

In Fig. 7 I, III, the blackened areas have been drawn to represent a hypothetical example of the extent and duration of the increased systemic output caused by the action of adrenaline upon the cardio-pulmonary system.

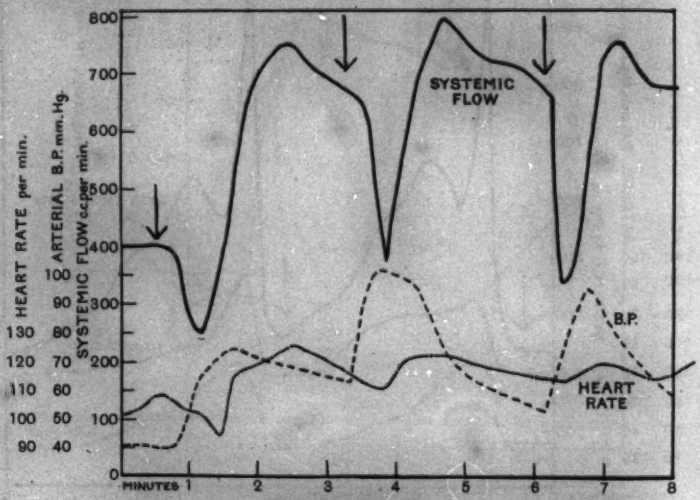

Fig. 5. Dog. Brain destroyed. The arrows mark times at which adrenaline was injected into the aorta.

The shaded areas represent a hypothetical example of the extent and duration of the influence of adrenaline upon the peripheral resistance.

Diagram I is an imaginary illustration of the course of events after an injection of adrenaline into the aorta. The influence of adrenaline upon the peripheral resistance precedes its influence upon the cardio-pulmonary system. In diagram II the algebraic sum of these changes in the flow has been plotted. The shape of the curve is essentially the same as that of the curves obtained for the aortic injections in the animal (Figs. 3, 5).

Diagram III is an imaginary illustration of the course of events after the intravenous injection of adrenaline. The influence of the adrenaline upon the cardio-pulmonary system precedes its influence upon the peri-

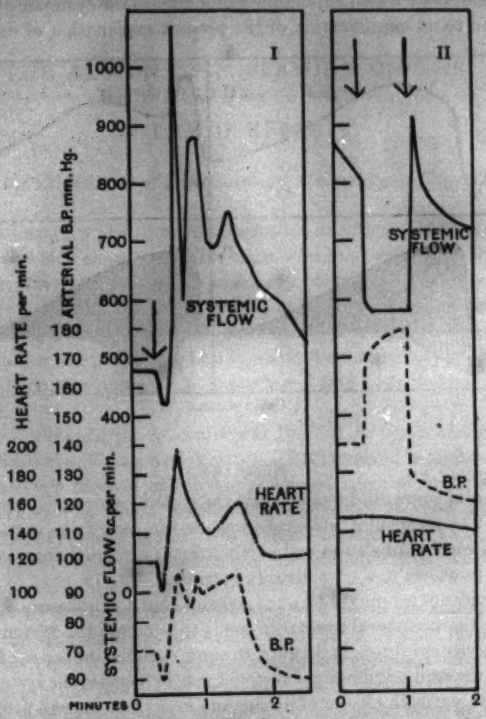

Fig. 6.

I. Preparation embodying artificial peripheral vascular system. The arrow shows time at which adrenaline was injected into the artificial peripheral vascular system.

II. Animal. During the time between the arrows the systemic flow was partially obstructed.

pheral resistance. In diagram IV the algebraic sum of these changes has been plotted. The shape of the curve is essentially the same as the shape of the curves obtained after intravenous injections in the animal (Figs. 2, 4).

Differences in the extent and duration of the influence of adrenaline

upon the peripheral resistance and upon the cardio-pulmonary system in different animals appear to be responsible for determining the extent to which the output is affected. Further experiment may establish additional factors by which adrenaline can influence the systemic output, and may lead to an amplification of the present explanation of its action.

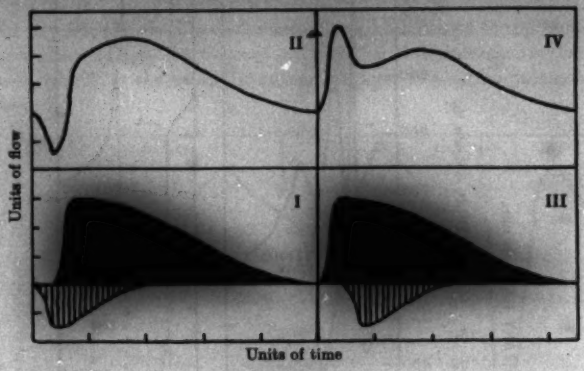

Fig. 7.

SUMMARY.

1. The influence of adrenaline upon the systemic output has been studied in dogs. The mechanical stromuhr was used to record the systemic flow.

2. In some animals the systemic output is not much increased or decreased, in others it is considerably increased.

3. Evidence is brought forward to show that the influence of adrenaline upon the peripheral resistance tends to decrease the systemic flow.

4. Evidence is brought forward to show that the influence of adrenaline upon the cardio-pulmonary system tends to increase the systemic flow.

5. The variable results of adrenaline injections appear to be due to variations in the extent of its influence upon the peripheral resistance and upon the cardio-pulmonary system.

I am very grateful to Prof. G. V. Anrep for suggesting the subject of this research.

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612.215.3:547.781.5

THE ACTION OF HISTAMINE ON THE RESPIRATORY TRACT.

By DAVID EPSTEIN.

(*From the Department of Pharmacology, University of Cape Town.*)

Dale and Laidlaw [1910] have shown that injections of histamine produce effects which closely resemble those seen in anaphylactic shock and asthmatic attacks. One of the chief symptoms is obstruction of the respiratory passages, and this is usually attributed to constriction of the bronchi or bronchioles. The obstruction occurs in varying degree in most animals, and in the guinea-pig it is so marked a feature as to cause death from asphyxia, the lungs at post-mortem being usually found greatly distended.

While working in the laboratory of Prof. J. A. Gunn at Oxford the author [Epstein, Gunn and Virden, 1932] evolved a modification of Trendelenburg's method of recording isolated rings of bronchi [Trendelenburg, 1912]. These preparations responded to most autonomic drugs in the same way as the plain muscle of the intestine. Histamine, however, failed to cause constriction of the bronchial rings of the cat and under certain conditions actually produced relaxation.

In view of this apparently conflicting evidence of the action of histamine on the bronchi it was decided to carry out a detailed investigation into the action of the drug on the respiratory passages. Since the responses to peptone more closely resemble the symptoms of anaphylactic shock and asthma than those produced by histamine, some experiments have also been performed with this drug. A preparation of peptone manufactured by Morson and Son was employed. Most of the work has been carried out on cats and guinea-pigs, but a few experiments have also been done on rabbits. Freshly prepared solutions of ergamine acid phosphate (B.W. and Co.) were used in the experiments and the doses refer to this salt, *e.g.* "1 mg. of histamine" means 1 mg. of this preparation.

Experimental methods and results.

I. *Lung volume records.*

The lung volume changes in the cat were recorded by means of sternal- and lung-shields as described by Jackson [1913, 1914, 1917]. Voluntary respiration was abolished by destroying the medulla by plunging a sharp instrument through the atlanto-occipital membrane and plugging the wound immediately afterwards with cotton-wool. (Previously both vagi had been cut and both carotid arteries ligatured under ether.) Artificial respiration was immediately started.

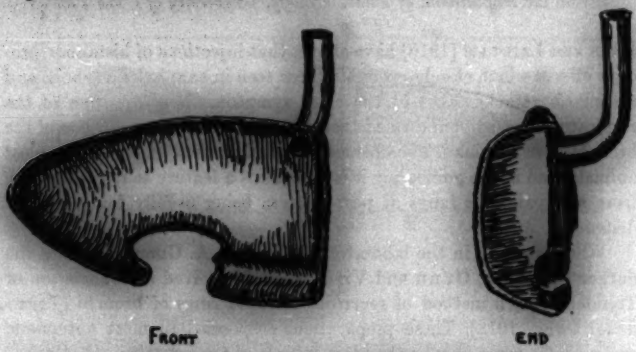

Fig. 1. Lung-shield (after Jackson) with a metal side tube for connecting to a tambour. (See text.)

The sternal-shield method of registering the lung volume changes requires very little manipulation, but unfortunately the records are complicated by the volume changes of the heart. The lung-shield method, however, gives good results provided it is carefully placed in position so as not to damage or compress the vessels at the root of the lung. When the shield is in position Jackson records the lung changes by making another opening in the chest wall, through which is passed a tube connected to a tambour. The latter step was obviated by soldering a hollow metal tube to the shield at the junction of the mediastinal and diaphragmatic surfaces close to the anterior border (Fig. 1). The tube (bent at right angles) projected through the original incision in the chest wall and was connected by a rubber tube to a tambour. The metal tube was clamped to a stand to keep the shield in the desired position. Positive pump pressure

was used to inflate the lungs, the pump having been connected to a cannula in the trachea.

Intravenous injections of histamine caused a definite diminution in the lung volume with doses of 0·1 mg. per kg. But even with much larger doses it was often impossible to cause complete occlusion. For example, in Fig. 2 a dose of 5 mg. per kg. of histamine caused a marked but incomplete obstruction which was sufficient, however, to bring on symptoms of

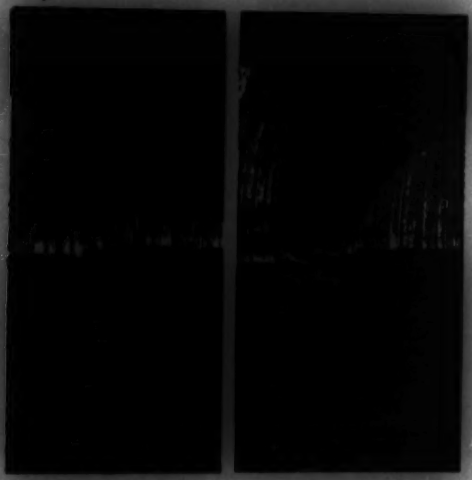

Fig. 2. Cat. Brain pithed. Lung volume. Shows marked but incomplete obstruction of air passages after 5 mg. per kg. histamine. Arecoline (0·25 mg. per kg.) produced complete air obstruction, which was partially abolished by atropine.

asphyxia, a fatal termination being prevented by mechanically dilating the bronchi. In contrast with these results complete diminution of volume could easily be produced by small injections of arecoline (Fig. 2).

Changes in lung volume similar to those in the cat were produced in pithed rabbits. It was found that the blood-pressure of these animals, however, was not well maintained.

Guinea-pigs were given doses of paraldehyde (2 c.c. or more per kg.) intraperitoneally in order to paralyse the respiratory centre. The lung volume was recorded by simply making a small incision in the chest wall

so that a thistle funnel could be squeezed in. The aperture was then rendered airtight and the tube connected to a tambour. The injections of histamine produced powerful effects, even such small doses as 0·01 mg. per kg. sometimes causing complete obstruction to the exchange of air in the lungs (Fig. 3). In one experiment, in which voluntary respiration had been abolished by paraldehyde, histamine caused the onset of inspiratory movements, as if the drug had stimulated the respiration.

Fig. 3. Guinea-pig. Respiratory centre paralysed by paraldehyde. Shows complete air obstruction after 0·1 mg. per kg. histamine. Repeated injections of adrenaline failed to relieve the effect and animal died of asphyxia.

From the experiments described above it is seen that histamine causes obstruction to the passage of air into or out of the lungs of the cat, rabbit and guinea-pig. The guinea-pig is much more susceptible than the cat or rabbit, small doses sufficing to cause complete obstruction. In the cat large doses also cause a certain degree of occlusion, but even very large doses often fail to produce complete obstruction. These results are in close agreement with the findings of Dale and Laidlaw [1910]. Similar effects were seen with peptone, *e.g.* 0·1 gm. of peptone per kg. also produced partial occlusion of the air passages of a cat.

The obstruction in the respiratory tract described above is usually attributed to constriction of the bronchi or bronchioles. The lung volume

records, as obtained above, however, are complicated by circulatory changes in the lungs under observation, and these pulmonary vascular changes may be important factors in causing obstruction in the respiratory passages. In order to determine the relative importance of (1) the vascular changes in the lung, and (2) the direct action of the drugs on the bronchi, in causing obstruction in the respiratory passages, further experiments were carried out.

II. *Isolated rings of trachea or bronchi.*

Trendelenburg [1912] introduced the method of investigating the direct reactions of the bronchi by means of the isolated bronchial ring preparation. He employed the bronchi of the ox, carefully dissecting away the cartilage and suspending the remaining plain muscle in a bath of Ringer's solution warmed to body temperature. The lever had to be weighted fairly heavily to remove the marked degree of tone usually present in the muscle. Macht and Ting [1921] carried out a series of investigations on the bronchi of the pig, following the procedure of Trendelenburg very closely. Titone [1913] investigated the isolated bronchi of the calf, sheep, pig and dog, merely suspending the rings without previously dissecting away the cartilage as described by Trendelenburg. The excessive tone, however, had again to be abolished by weighting. Golla and Symes [1913] investigated an isolated muscle strip of the cat's trachea, although they showed no tracings of these experiments. Franklin [1926] used an isolated ring of a sheep.

In the present investigation a number of isolated rings of the trachea or bronchi have been strung together side by side like the wheels of a tandem bicycle. According to the diameter of the rings it has been usual to employ from 7 to 14 rings, which together with a lever magnification of 12 to 16 times has magnified the reactions of each ring approximately 100 to 200 times. Using this method the author has been able to record isolated rings of the trachea or bronchi of the cat, rabbit and guinea-pig.

After the animal had been killed by a blow on the back of the neck and bled by severing the vessels in the neck, the trachea was carefully dissected out and cut into sections to give rings of various widths. In the cat the diameter of the trachea was usually 5 to 10 mm., and fairly wide rings were cut, *e.g.* about 6 mm. In the case of the guinea-pig, however, the diameter of the trachea was very much smaller (about 3 to 4 mm.), and so rings having a width of about 3 to 4 mm. were used. Also, the bronchi as they passed into the lungs were carefully dissected out. These tubes which had an average diameter of about 1·5 mm. were carefully

cleaned of all lung tissue, cut into sections of about 3 mm. wide and then strung together tandem fashion, using a needle and thread.

When the rings of trachea or bronchi were tied together the string was made to encircle the cartilaginous portions of the rings, when possible, so as to avoid tearing the intercartilaginous strips of plain muscle.

The preparations were then suspended in a bath of Ringer's solution maintained at 37° C., one end being fixed to a glass rod and the other end attached to a lever by means of a thread. Air was bubbled through the Ringer's solution (NaCl 0·9 p.c., KCl 0·042 p.c., $CaCl_2$ 0·022 p.c., and $NaHCO_3$ 0·02 p.c.) at a constant rate. In contrast with the methods of Trendelenburg and Macht, in which heavy weighting of the lever was necessary to overcome the excessive tone of the preparations, in the present method very lightly balanced levers were required. The rings have never shown spontaneous movements when set up in Ringer's solution, but the addition of certain drugs has sometimes produced irregular rhythmic movements. These effects, together with the responses of the rings to other drugs, will be described in another paper.

It was found that the ring preparations were normally fully relaxed, so that the actions of broncho-constrictor drugs could be studied by merely adding the drugs to the baths containing the preparations. Constriction of the rings had first to be produced, however, before any effect could usually be obtained with broncho-dilator drugs. To obtain the preliminary constriction in these cases it was found that arecoline and acetyl choline were both suitable, because their dosage could be graded to give effects which were quite definite but which would not mask the relaxing effects of broncho-dilator substances when given subsequently in sufficient concentration. When at rest the preparation usually gave horizontal straight lines, so that the action of constrictor drugs could be easily seen. When the effects of various drugs had been produced the record again usually consisted of a horizontal line, so that the actions of additional substances were again fairly obvious.

(*a*) Using ring preparations of the cat's trachea or intrapulmonary bronchi it was found that constriction was produced by the parasympathetic group of drugs, and relaxation by the sympathetic group [Epstein, Gunn and Virden, 1932]. Histamine acid phosphate, however, failed to constrict the rings when given in concentrations ranging from 1 in 1,000,000 to 1 in 13,000. In Fig. 4 the concentration has gradually been increased, but each concentration has been tried on previously untreated preparations in other experiments (Fig. 5).

In the course of the investigation it was found that after histamine

arecoline often failed to constrict the rings in concentrations which had been active when given previous to the histamine (Fig. 5). The action of

Fig. 4. Reduced to ½ of original size. Cat. Isolated tracheal rings (7). Concentrations of histamine (1 in 1 million; 1 in 300,000; 1 in 100,000 and 1 in 13,000) all had no effect on the fully relaxed preparation. Arecoline (1 in 1 million) however produced a powerful contraction.

Fig. 5. Reduced to ½. Cat. Isolated tracheal rings (8). Histamine (1 in 100,000) had no apparent effect on the fully relaxed preparation. Arecoline in a concentration of 1 in 8 million failed to have any effect in the presence of histamine (compare with the effect shown later in the figure when histamine has been washed out); but increasing the concentration to 1 in 2 million caused a powerful contraction. Replacing the solution with Locke's solution (twice) caused the rings to relax gradually. The rings were now constricted by arecoline in concentrations of 1 in 20 million and 1 in 10 million. Histamine (1 in 100,000) produced a marked relaxation of the contracted rings, and adrenaline (1 in 3 million) caused a further relaxation.

histamine was therefore tested after the constriction produced by weak concentrations of arecoline or acetyl choline. Under these conditions histamine often produced relaxation of the cat's tracheal or bronchial

rings (Figs. 5 and 6). The arecoline and acetyl choline contraction was only partially abolished by histamine, and stronger concentrations of arecoline or acetyl choline were able to cause a further degree of constriction. It may be claimed that the relaxation seen with histamine is

Fig. 6. Reduced to ½. Cat. Tracheal rings (7). Contraction with acetyl choline (1 in 12 million) and relaxation with histamine (1 in 100,000). Further relaxation on replacing solution with Ringer's fluid.

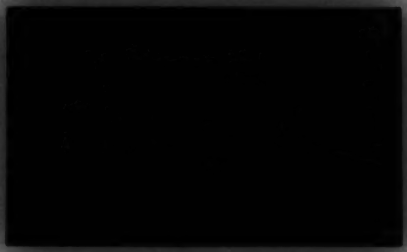

Fig. 7. Reduced to ½. Cat. Intrapulmonary bronchi (12 rings). Histamine in a strength of 1 in 1 million had no effect; 1 in 100,000 produced a slight relaxation; 1 in 15,000 had no effect. Arecoline (1 in 400,000) caused a contraction, which gradually passed off when replaced by fresh Ringer's solution (L.S. 2). Time in 10 seconds. The time signal was lowered at X.

due to the drug having a weak atropine-like effect and being able in certain proportions to abolish the effects of parasympathetic drugs like arecoline. However, in two experiments when the untreated rings were apparently not fully relaxed and still retained a slight degree of tone, concentrations of histamine of 1 in 100,000 or stronger produced a slight relaxation (Fig. 7). Further experiments were also carried out with

barium chloride. The constriction produced by barium was usually complicated by the occurrence of irregular rhythmic waves, so that the effects of subsequent drugs were sometimes difficult to interpret. It was found, however, that if given in strong concentrations, *e.g.* 1 in 10,000, histamine

Fig. 8. Same experiment as in Fig. 7. Peptone in concentrations of 1 in 1 million; 1 in 100,000 and 1 in 20,000 had no effect on the fully relaxed rings. Arecoline (1 in 400,000) caused contraction, adrenaline (1 in 500,000) produced relaxation and atropine (1 in 1 million) produced a further lowering of tone to normal.

Fig. 9. Reduced to ¼. Guinea-pig. Isolated tracheal rings (8). Arecoline (1 in 1 million) caused a contraction, which was unaffected by washing out with Ringer's solution (L.S.). Adrenaline (1 in 2 million) produced relaxation. Drugs washed out and replaced by fresh Ringer (L.S.). Lever raised. At *C* 0·5 c.c. of Ringer's solution was added to the bath. This was repeated but no effect was produced in either case. These served as controls. At *H* histamine was added to give a concentration of 1 in 300,000. This caused constriction of the rings, which disappeared on washing out with fresh Ringer (L.S.).

sometimes produced a weak relaxation after barium, or abolished the rhythmic waves. Under these conditions the strong concentrations of histamine occasionally gave a preliminary transient weak contraction before showing the slight degree of relaxation.

Peptone in concentrations of 1 in 1 million to 1 in 20,000 also failed to contract the cat's bronchi (Fig. 8), and the strong concentrations relaxed the tissue if previously constricted with arecoline.

(*b*) With the tracheal rings of the rabbit histamine again failed to cause constriction when given in concentrations of 1 in 4 million to 1 in 200,000. Histamine occasionally produced relaxation after the contraction caused by other drugs.

(*c*) In contrast with the results in the cat and rabbit it was found that the tracheal rings of the guinea-pig were constricted by histamine. Concentrations of 1 in 1,000,000 to 1 in 75,000 were used (Figs. 9 and 10). In one experiment performed on rings of the bronchi the effects were poor, but a weak constriction was again obtained with histamine.

In connection with these results it is of interest to note that Trendelenburg [1912] failed to obtain any effect with histamine on the isolated bronchi of the ox, Macht and Ting [1921] described contraction of the isolated bronchi of the pig, Titone [1913], working with bronchial rings of the calf, sheep, pig and dog, also recorded concentrations with histamine, but Golla and Symes [1913] obtained no effect on the trachea of the cat.

Fig. 10. Reduced to ½. Guinea-pig. Tracheal rings (7). At *A* histamine (1 in 1 million) caused constriction. At *B* increasing the concentration to 1 in 300,000 produced a further constriction. At *C* tyramine acid phosphate (1 in 100,000) caused an insignificant degree of relaxation. At *D* adrenaline (1 in 1 million) caused complete relaxation.

(i) From the results obtained above it would appear that the obstruction of the respiratory passages, that occurs in the guinea-pig after histamine, is due to a direct stimulant action of the drug on the musculature of the respiratory tract. As shown above, constriction of the trachea and bronchi appears to take a definite part in this action, and probably constriction of the bronchioles also occurs.

(ii) On the other hand, although it has been shown that histamine also causes obstruction of the respiratory passages of the cat [Dale and Laidlaw, 1910, and others—see also Section I], the experiments described above indicate that this drug does not cause contraction of the trachea and intrapulmonary bronchi but may actually relax these structures. The obstruction of the respiratory tract in the cat with histamine must therefore be due to a direct constriction of the bronchioles by the drug, or it is possible that the changes in pulmonary circulation that occur with histamine cause a mechanical compression of the respiratory tubes. In order to decide these points some experiments were performed with histamine on the pulmonary circulation of the cat.

III. *Pulmonary circulation.*

Dale and Laidlaw [1910] showed that histamine caused a fall in the systemic blood-pressure in the cat, but produced a marked rise in the pulmonary blood-pressure, the latter effect being attributed to constriction of the pulmonary arterioles. Dixon and Hoyle [1930] claimed that histamine produced "congestion of the whole pulmonary circulation," for they found that there was an increase in the blood volume in the lung and an increased blood flow through the lung, these changes leading to a rise in pulmonary pressure.

Other investigators have shown that vascular congestion of the lungs produces obstruction of the respiratory passages. Drinker and Agassiz [1925] produced congestion of the pulmonary circulation by clamping the pulmonary veins of the cat. This caused a reduction in the amount of air that could be pumped into the lung, but the condition returned to normal again when the vein clamp was removed, showing that the changes had not been due to the formation of œdema in the lungs. They concluded that the obstruction to air entrance produced by pulmonary vascular congestion was "feeble in the normal non-œdematous and non-fibrotic lung tissue of the cat." By taking measurements on some of the graphs in this paper the author found that the reduction in the amount of air passing into the lungs never exceeded 25 p.c. of the total even during the severest degrees of pulmonary congestion.

Hanzlik [1925] clamped the pulmonary veins of the guinea-pig and found that the cast obtained by pouring celloidin into the trachea and bronchi of the excised lungs was markedly reduced, indicating that obstruction of the respiratory passages had occurred, presumably by vascular compression of the bronchioles.

To determine the part played by the pulmonary vascular congestion seen with histamine in causing obstruction of the air passages, simultaneous records of the pulmonary blood-pressure and the lung volume were taken in cats. The animals were anæsthetized with intraperitoneal injections of paraldehyde, a dose sufficient to paralyse the respiratory centre (usually about 2 to 2·5 c.c. per kg.) being used. Artificial respiration was carried on by a pump attached to the trachea, positive pressure being again employed. The volume of one of the lower lobes of the right lung was recorded by cutting away the ribs on the right side of the chest and enclosing the lobe in an oncometer after the manner of Dixon and Brodie [1903]. The oncometer used in the present experiments consisted merely of a wide glass cylinder with a narrow, oval-shaped orifice at one

end, the other end being tightly corked; a tube passing through the cork connected the apparatus with a tambour. As shown by Dixon and Brodie, handling of the lung produces a collapsed organ which fails to distend adequately under pump pressure. It was found that by placing the oval-shaped orifice of the oncometer in contact with the lung and applying suction at the other end of the apparatus, the lung could be sucked into position in the oncometer with the least possible damage. The oval-shaped orifice was then rendered airtight by blocking it with vaseline or by clamping the apparatus at an angle fairly tightly into the chest cavity. The pulmonary blood-pressure was recorded at the same time by placing a cannula into the branch of the pulmonary artery supplying the middle lobe of the left lung, half-saturated sodium sulphate being used in the manometer. The systemic blood-pressure was recorded by means of a cannula in the left carotid artery and the drugs were injected slowly into the right femoral vein.

Fig. 11 illustrates the results of such an experiment. The injection of 0·2 mg. per kg. of histamine caused a marked rise in pulmonary blood-pressure (75 mm.) and a fall in systemic blood-pressure. The lung volume was at the same time diminished, indicating that marked obstruction had occurred in the respiratory passages. The respiratory obstruction commenced about 10 seconds after the beginning of the rise in pulmonary blood-pressure and both reached a maximum about the same time. The pulmonary pressure returned to normal after 78 seconds, but at this time marked obstruction of the air passages was still present, the record showing a 50 p.c. reduction in the amount of air that could be pumped into the lung. The lung volume returned to normal only after 4 minutes. These results show that the obstruction of the air passages produced by histamine can persist long after the congestion of the pulmonary circulation has passed off.

It was also found that after repeated injections of histamine both the systemic and pulmonary blood-pressures usually gave very feeble responses with the drug, but that marked obstruction of the air passages could still be produced at each injection of the substance (Fig. 12). These results indicate that histamine is able to cause obstruction of the air passages in the cat quite independently of any changes produced in the pulmonary circulation.

There was still the possibility that the pulmonary vascular congestion served as an accessory factor in producing the air obstruction seen with histamine. However, it was noted that the degree of air obstruction produced by the same doses of histamine was the same, whether there had

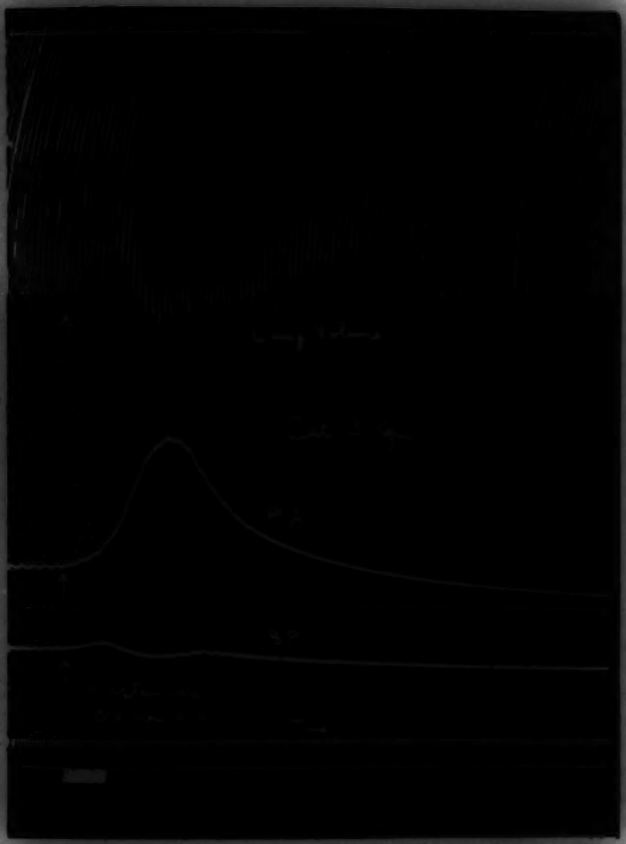

Fig. 11. Cat. The injection of 0·2 mg. per kg. of histamine produced a diminution of lung volume (upper record), a rise in pulmonary pressure (middle record) and a fall in systemic pressure (lower record). (See text.) Time in seconds.

been a marked rise in pulmonary pressure or whether (later on in the same experiment) the change in pulmonary pressure had been negligible. Thus in Fig. 12 an injection of 0·1 mg. per kg. of histamine produced an air obstruction of 35 p.c. although the rise in pulmonary pressure was only 5 mm.; while the same dose of histamine given earlier in this experiment produced the same degree of air obstruction but the rise in pulmonary pressure was 25 mm. Again, in another experiment an injection of 0·05 mg. per kg. of histamine caused a rise in pulmonary pressure of 35 mm. of half-saturated sodium sulphate solution, and there was a reduction in the passage of air of only 7 p.c. Later on in the experiment the same degree of air obstruction occurred with this dose of histamine, but there was a rise in pulmonary pressure of only 8 mm. These experiments suggest that any accessory value that the pulmonary vascular changes may have in increasing the air obstruction seen with histamine is probably insignificant.

Fig. 12. Same experiment as in Fig. 11 after repeated injections of histamine. Shows that a further injection of histamine (0·1 mg. per kg.) has a negligible effect on the pulmonary blood-pressure, but is still able to produce a diminution of lung volume.

Dale and Laidlaw [1910] claim that histamine increases the bronchial secretion and this might tend to cause some obstruction of the respiratory tract, which would persist until the passages had again been cleared. In many of the experiments, however, the lung volume records showed that the obstruction of the air passages passed off completely if the doses of histamine had been small and had not been repeated too frequently, indicating that any increase in bronchial secretion played a relatively unimportant part in causing respiratory obstruction.

Sometimes with large frequently repeated doses, however, the obstruction passed off only partially, even if atropine had been previously given to prevent any possible increase in bronchial secretion. The persistence of the obstruction in these cases may perhaps be partly explained by assuming that a certain amount of œdema of the lungs had occurred

as a result of the repeated pulmonary vascular congestion, and that this was causing a partial mechanical obstruction of the respiratory passages. It is also possible that an angioneurotic œdema of the respiratory mucous membrane, and especially of that portion lining the bronchioles, may be the cause of air obstruction seen with histamine. However, if such were the case, one would expect the obstruction to persist for some considerable time, whereas it has been shown that with small doses of histamine the condition often passes off completely fairly soon. In the more persistent air obstruction seen after large frequently repeated doses of histamine it is possible that this condition may be present and serve as an accessory factor.

From the experiments described in this section it has been shown that histamine can cause obstruction of the respiratory passages of the cat quite independently of any changes in the pulmonary circulation and of changes in the amount of bronchial secretion. While the accessory value of the pulmonary vascular congestion in increasing the obstruction is insignificant, it is possible that this congestion when frequently produced may in certain cases be followed by some pulmonary œdema, which may mechanically impede to some extent the passage of air through the respiratory passages. It is also possible that increased bronchial secretion produced by histamine may in certain cases serve mechanically as an accessory factor in increasing the air obstruction.

IV. *Perfusion of the lungs through the respiratory passages.*

Baehr and Pick [1913] and McDowall and Thornton [1930] have investigated the responses of bronchi by perfusing drugs through the pulmonary vessels. To eliminate any possible vascular effects a method of direct perfusion of the drugs through the respiratory passages, based on the method described by Sollmann and von Oettingen [1928], was employed in the present investigation.

The animals were killed by a blow on the back of the neck and bled by severing the vessels in the neck. The lungs and trachea were excised intact, placed in a large dish of Ringer's solution and gently massaged until as much air as possible had been squeezed out. Both lungs of the guinea-pig were perfused by tying a cannula into the trachea just above its bifurcation; in cats only one lung was perfused, the cannula being tied into the main bronchus. The preparations were now connected to the perfusing apparatus (Fig. 13) and sufficient Ringer's fluid allowed to flow in to distend the lungs slightly. The lungs were massaged again to squeeze out any remaining air, the bubbles being allowed to escape through a side

tube just above the cannula. The side tube was then closed and sufficient Ringer's solution allowed to run in to distend the lungs moderately. A number of shallow punctures was made with a needle through the surface of the lungs, especially in the neighbourhood of the borders of the lungs. The fluid was thus able to pass through the trachea, bronchi and bronchioles, and escape through the punctured alveoli. The height of the Mariotte bottle was arranged so that the pressure of fluid was just sufficient to distend the lungs to about their normal size. In the case of the

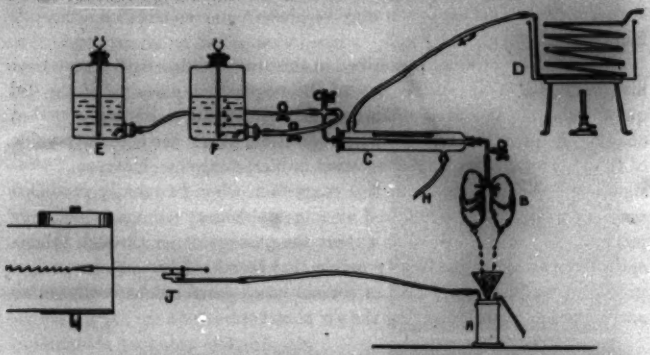

Fig. 13. Showing arrangement of apparatus used for perfusing the respiratory passages. A = Gunn's siphon-recorder; B = lungs with threads attached so that fluid collects in funnel; C = Gunn's warming jacket; E = Mariotte bottle containing Ringer's solution; and F = bottle with a definite concentration of drug to be investigated. The rubber tubes connecting E and F to C should be of equal length and should be relatively shorter than shown. The heights of the bottles should be as indicated in the text.

cat it was found that the lower end of the tube in the Mariotte bottle should be about the same level as the tip of the cannula in the bronchus. The lowest point of the dependent lung was usually about 6 cm. below the cannula tip, so that the perfusion height in these cases was about 6 cm. Under these conditions excellent results were obtained, but if the perfusion pressure was increased the lungs became over-distended and even strong concentrations of arecoline, etc., had no appreciable effect. In guinea-pigs, however, it was found that greater perfusion heights could be used, *e.g.* good results were obtained with a total height of 15 cm. Sollmann and von Oettingen measured the inflow by counting or recording the air bubbles entering the Mariotte bottle. In the present

experiments it was found more convenient to measure the outflow, and typical results could be obtained even after the preparation had been up for 1½ to 2 hours. The outflow was recorded by Gunn's siphonometer [Gunn, 1913 *b*]. To collect the fluid from such a large surface as the cat's lung a wide funnel could be used on the siphon-recorder or by attaching threads to the projecting lowest points of the lung the fluid could be made to collect in the mouth of the funnel. The upper lobe of the cat's lung usually failed to distend with fluid, probably because the cannula had been tied in beyond the orifice of the bronchus to this lobe. To eliminate any possibility of fluid occasionally being able to enter this lobe and so produce erroneous results, the lobe was tied off at the beginning of the experiment. The perfusing fluid was warmed to 37° C. by means of Gunn's apparatus for perfusing the isolated mammalian heart [Gunn, 1930 *a*]. Since the perfusion height required was not very great, it was found more convenient to have the warming jacket in the horizontal plane (or with a very slight incline) than in the vertical position. This eliminated having to use long portions of rubber tubing connecting the Mariotte bottles to the warming jacket, and when changing perfusing fluids the drugs in the Mariotte bottles were able to reach the lung very quickly. When the outflow was constant under perfusion with Ringer's fluid, a solution of the drug to be investigated was allowed to flow in from another Mariotte bottle, keeping the perfusion pressure unaltered. In these experiments the exact strength of the drug employed was known. In other experiments 1 c.c. of a concentrated solution of the drug was injected into the rubber tube just above the lung cannula.

Histamine produced marked diminution in the outflow from the lungs of both animals, but the effects in the guinea-pig were many times more powerful than those in the cat. A concentration of 1 in 5 million histamine caused a definite obstruction to the flow of fluid through the trachea and lungs of the guinea-pig. A strength of 1 in 500,000 produced almost complete obstruction, the outflow being reduced from 18 c.c. per minute to 0·4 c.c. per minute (Fig. 14, lower record). In the upper record of the same figure an injection of 1 c.c. of a 1 in 2000 solution of histamine into the perfusing fluid reduced the outflow from 45 c.c. to 5 c.c. per minute, adrenaline (1 c.c. of 1 in 1000) causing a great increase again.

Weak effects were seen with solutions of 1 in 500,000 histamine in the cat's lung, but with 1 in 100,000 marked obstruction occurred, the outflow being reduced from 9 c.c. to 1 c.c. per minute (Fig. 15, upper record), but this was increased again by an injection of 1·5 c.c. of adrenaline (1 in 1000) and by later changing over to Ringer's solution. An injection of

1 c.c. of a 1 in 1000 solution of histamine reduced the outflow from 13 c.c. to 2 c.c. per minute (lower record). Weak effects were also seen with peptone.

Fig. 14. Reduced to $\frac{1}{3}$. Guinea-pig. Lung perfusion record. Each notch represents an outflow of 3 c.c. Time record shows intervals of 10 seconds. Lower record: at the arrow perfusion was changed from Ringer's solution to a 1 in 500,000 solution of histamine, which resulted in almost complete obstruction to the outflow. Upper record: the injection of 1 c.c. of a 1 in 2000 solution of histamine into the rubber tube just above the tracheal cannula caused a marked diminution in outflow. The injection of 1 c.c. of adrenaline (1 in 1000) greatly increased the outflow.

Fig. 15. Reduced 70 p.c. Cat. Upper record: at the first arrow the perfusing fluid was changed over from Ringer's solution to a 1 in 100,000 solution of histamine, and a marked diminution in outflow occurred. At the second arrow an injection of 1·5 c.c. of adrenaline (1 in 1000) was given, which increased the outflow even though the histamine solution was still being perfused. At the next arrow (L.S.) the perfusion was switched back to Ringer's solution. The lower record shows the effect of injecting 1 c.c. of a 1 in 1000 histamine solution and then the result of giving 1 c.c. of 1 in 2000 adrenaline.

These experiments show that histamine can cause obstruction of the respiratory passages of the cat and guinea-pig by a direct action on the respiratory tract, and the effect can thus occur independently of changes produced in the circulation and of other factors. This confirms the findings of Section III.

Discussion.

(*a*) *Guinea-pig.* By means of lung-volume records it has been confirmed that histamine is able to produce obstruction of the respiratory passages of the guinea-pig. By perfusing histamine solutions through the respiratory tract of excised lungs any effects on the pulmonary circulation were eliminated, and the fact that the obstruction was still produced under these conditions indicated that the drug must have a direct action on some portion or portions of the respiratory tract. Using ring preparations of the isolated trachea and bronchi it has been shown that histamine is able to constrict these structures to a marked degree. The air obstruction seen after injections of histamine in the guinea-pig can thus be explained wholly or chiefly by the direct constrictor effect of the drug on the plain muscle of the trachea, bronchi and probably the bronchioles too, *i.e.* by contraction of the musculature of the whole respiratory tract, or at least, the greater portion of it. Schultz and Jordan [1911] have shown that the mucous membrane of the secondary bronchi of the guinea-pig normally projects into the lumen in the form of folds, so that even a slight narrowing of the lumen will suffice to occlude these bronchi completely. This factor, taken in conjunction with the findings in this paper, viz. that the greater portion of the respiratory tract is contracted with histamine, would serve to explain the severity of the obstruction seen in guinea-pigs. The occlusion of the respiratory tract is often so marked that the condition frequently terminates in death from asphyxia.

(*b*) *Cat.* Using lung-volume records it has been confirmed that histamine is able to cause obstruction of the respiratory tract of the cat. By recording at the same time the pulmonary blood-pressure it has been shown that the air obstruction seen with histamine can occur quite independently of any changes in the pulmonary circulation. Any accessory value of the pulmonary congestion in increasing the air obstruction is probably unimportant. The obstruction can still occur, even if an increase in bronchial secretion is prevented, and also in the absence of pulmonary œdema. The obstruction would therefore appear to be due to a direct action of the drug on some portion of the musculature of the respiratory tract. This has been confirmed by directly perfusing the drug through the bronchial tree, thereby eliminating the circulatory and other factors. Using ring preparations of the trachea and intrapulmonary bronchi it has been found that histamine fails to constrict these structures and may under favourable circumstances actually relax them. Since it has been shown above that the air obstruction with histamine is due to a direct

action of the drug on some portion of the respiratory tract, it must be assumed that the more peripheral portions of the respiratory passages, namely the bronchioles, are constricted by the drug. This then is the chief cause of the respiratory obstruction seen in cats after histamine, although other factors may occasionally serve to increase the effect.

Obstruction of the air passages is also one of the chief symptoms seen in anaphylactic shock, and the guinea-pig again is much more affected than the cat. In this and in other respects the symptoms of anaphylactic shock very closely correspond to those produced by histamine, and the author therefore suggests that the air obstruction in both conditions can be explained on the same basis, viz. by an active constriction of the trachea, bronchi and probably bronchioles in the guinea-pig, and by constriction of only the bronchioles in the cat. Experiments on anaphylaxis are being carried out to decide these points.

In man the anaphylactic response is seldom fatal, *i.e.* it is relatively weak when compared with the severe reaction produced in the guinea-pig [see Sollman, 1926, p. 483]. In other words, the anaphylactic response in man resembles that seen in the cat rather than that of the guinea-pig, and it is possible that the air obstruction in this condition in man may prove to be due, as in the cat, to a constriction limited to the bronchioles. It may be found that this hypothesis also serves to explain the air obstruction that occurs in the closely related asthmatic attacks of man.

It is of interest to compare the actions of histamine on the respiratory passages of the cat as described above with the effects of the drug on the blood vessels of this animal [Dale and Richards, 1918]. Thus, while the trachea and bronchi are relaxed by histamine, the bronchioles or finer tubules are constricted; whereas, while the arterioles are constricted by histamine, the capillaries or finer vessels are relaxed. In both cases, however, the peripheral effects of the drug completely overshadow the other actions. It is of interest also to note that the only other mammalian tissue which is relaxed by histamine is the rat's uterus [Guggenheim, 1912].

Summary.

Using various methods of investigation it has been shown that histamine produces obstruction of the respiratory passages of the guinea-pig by a direct constrictor effect on the trachea and bronchi. The bronchioles probably also take part in the action. In the cat histamine causes obstruction chiefly by a direct constrictor effect on the bronchioles. The bronchi and trachea appear to take no part in the obstruction, for they may actually be relaxed by histamine.

A method has been described for recording the effects of drugs on isolated ring preparations of the trachea and bronchi of the commonly employed laboratory animals, viz. the cat, rabbit and guinea-pig.

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"CHLORIDE SECRETING CELLS" IN THE GILLS OF FISHES, WITH SPECIAL REFERENCE TO THE COMMON EEL.

By ANCEL KEYS[1] and E. N. WILLMER.

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Introduction.

The recent discovery of the chloride secretory activity of the gills of the eel [Keys, 1931*b*] demanded consideration of the histology of the gills. No anatomical basis was known to which the secretory activity might be ascribed and the view was firmly entrenched that respiratory epithelia in general are uniformly devoid of secretory elements. As Winterstein [1921, p. 256] puts it: "Nirgends ergibt sich ein morphologischer Anhaltspunkt für eine 'sekretorische' Tätigkeit, nirgends zeigt das respiratorische Epithel die charakterischen Eigenschaften sezernierender Zellen."

It was shown [Keys, 1931*b*; Bateman and Keys, 1932] that the chloride secretion exhibited by the gills of the eel in sea water is an active process requiring the expenditure of large amounts of energy. The possibilities would seem to be:

(1) A simple respiratory epithelial membrane capable, in the eel, of accomplishing an amount of osmotic work comparable, on the basis of equal weights of tissue, with that done by the mammalian kidney, or

(2) Special secretory cells present in the gills, implying error in the older studies.

The present paper is a report of investigations which revealed the undeniable existence of large numbers of secretory cells in the gills of eels and other teleosts. The constant appearance of great numbers of these cells so situated in the gills as to have access to both internal and external media, combined with the proof that they are not mucous cells, gives them an unusual physiological interest.

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Methods.

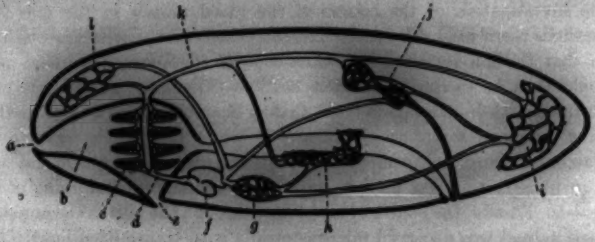
The macroscopic anatomy of the branchial circulatory system in the eel was studied by perfusion methods [Keys, 1931*a*] and by dissection of anæsthetized eels, pithed eels, and fixed and injected specimens. For the investigation of the course of the blood vessels in the gills four methods were used. The main efferent vessels of the gills were tied off so that the gill vessels, which thus became engorged with blood cells, could then be easily identified in sections. Secondly, the vessels were perfused with Indian ink dialysed against Ringer's fluid, and this method gave the clearest picture of the way in which the vessels traverse the filaments. Thirdly, the method of injection of silver nitrate and subsequent reduction was used to impregnate the endothelial lining of the vessels. Finally, the course of blood flow through the gills in the anæsthetized animal was observed microscopically with transmitted light.

The gills for microscopical investigation were fixed in Bouin's or Zenker's fluids or with Flemming's fluid from which the acetic acid was omitted. Attempts were made to fix the tissues by perfusion with 4 p.c. neutral formalin, but the results were unsatisfactory as a considerable amount of shrinkage occurred in the cytoplasm, especially in the cytoplasm of the secretory cells to be described. Sections were stained with hæmatoxylin and eosin or hæmalum and eosin, and these, after fixation in Bouin's fluid, produced the most satisfactory histological pictures. Mallory's triple stain and Giemsa's azure eosin were also used with success. A considerable number of sections were treated with metachromatic thionin to establish the positions of mucous cells. The finer structure of the cells was studied chiefly in cells fixed with Flemming's fluid (without acetic acid) and stained with Heidenhain's iron hæmatoxylin.

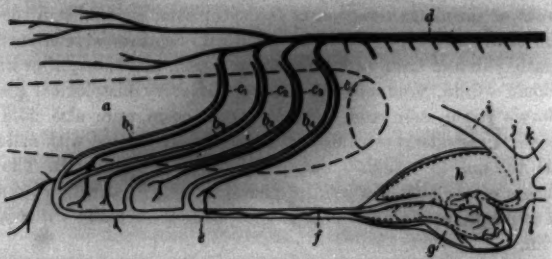
The anatomy of the branchial circulatory system.

The arrangement of the general and the branchial circulatory systems in the eel is typical of the teleost fishes. Text-fig. 1 is a diagrammatic representation of the general circulation in the teleost. Venous blood from the anterior and posterior cardinal systems passes through the ductus cuvieri and joins the blood from the hepatic vein in the sinus venosus. From there the blood traverses, in succession, the auricle, the ventricle, bulbus arteriosus, ventral aorta, the gills and thence to the dorsal aorta. The details of the circulation in the gills will be described later in this paper.

A less diagrammatic picture of the branchial circulation is given in Text-fig. 2 in which oxygenated blood is represented in black. The gills of the right side are not shown. The arterial supply to the tissues of the head is derived from small branches of the anterior efferent vessels of



Text-fig. 1. Essential circulatory system (schematic). *a*, mouth; *b*, branchial chamber; *c*, branchial vessels; *d*, ventral aorta; *e*, branchial aperture; *f*, heart; *g*, hepatic circulation; *h*, capillaries of alimentary canal; *i*, capillaries of posterior part of body; *j*, kidney; *k*, dorsal aorta; *l*, capillaries of anterior part of body.



Text-fig. 2. Branchial circulatory system. *a*, branchial chamber; b_1, b_2, b_3, b_4, afferent branchial vessels; c_1, c_2, c_3, c_4, efferent branchial vessels; *d*, dorsal aorta; *e*, ventral aorta; *f*, coronary artery; *g*, ventricle; *h*, auricle; *i*, anterior cardinal sinus; *j*, sinus venosus; *k*, posterior cardinal sinus; *l*, hepatic vein.

the gills, and the venous drainage from the head tissues is collected by the anterior cardinal system. We did not find a highly developed hypobranchial system such as is present in some elasmobranchs [Keys, 1928].

We have confirmed the description of the coronary system given by Grant and Regnier [1926]. The left coronary artery is shown, in Text-fig. 2, traversing the ventral aorta posteriorly to the heart; the

distribution of the vessels on the heart itself is extremely variable. As Grant and Regnier found, the coronary veins drain into the sinus venosus.

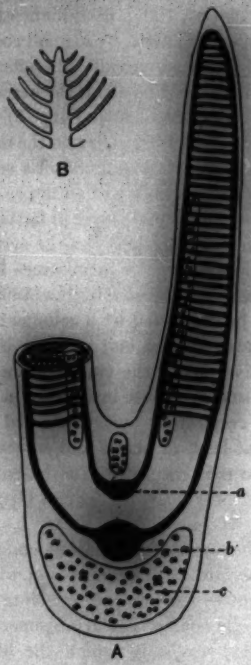


Fig. 3.

Fig. 4.

Text-fig. 3. A. Diagrammatic section through gill bar showing arrangement of vessels. *a*, afferent vessel; *b*, efferent vessel; *c*, cartilage. B. Diagram showing arrangement of leaflets at the tip of a filament.

Text-fig. 4. Diagrammatic section across a filament. *a*, mucous cells; *b*, afferent blood vessel; *c*, cartilage; *d*, leaflet cut parallel to its surface; *e*, efferent blood vessel; *f*, leaflets cut across.

The eel, like the other teleosts generally, possesses four branchial bars on either side, and each bar bears, on its outer side, a double row of filaments. Each filament, in turn, bears on each side a row of lamellæ or respiratory leaflets which provide the surface for both respiratory

and osmotic exchange. Text-fig. 3 shows a transverse section through a gill bar and also a diagram of the tip of a filament viewed at right angles to the gill bars.

As described by earlier workers [see Oppel, 1905], each filament on the bar is supported for about two-thirds of its length by a small rod of cartilage (the gill ray) which is situated on the inner side. The afferent branchial artery (which carries venous blood) courses along the external side of the gill bar in the V between the filaments (see Text-fig. 3) and sends a branch to each filament which runs along the gill ray on the inner side. At the level of each leaflet this filament arteriole gives off a small branch which opens into the leaflet. The leaflet consists of an upper and lower respiratory epithelium, each composed of a single layer of flattened cells; between the upper and lower respiratory epithelia lies the system of blood channels. The blood is enclosed between two membranes kept apart by somewhat columnar cells (the pilaster cells of Bietrix [1895*b*], see Oppel, p. 23 *et seq.*) as a roof might be supported by a colonnade of pillars. These supporting cells are expanded at each end and it is these expansions which, by fusing together, give rise to the two membranes. The nuclei of the supporting cells are situated almost invariably in the middle region of the cell. Between the respiratory epithelium and the blood channels there is some evidence, at least in places and especially towards the base of the leaflets, for the existence of a true basement membrane such as has been described by previous workers [see Oppel, 1905].

After the blood has flowed through the leaflet it is collected again on the outer side of the filament and flows into an efferent vessel which traverses the outer margin of the filament to the gill bar, receiving the blood from each leaflet which it passes in its course. The arrangement of the blood channels is illustrated in Text-fig. 4, in which the black represents blood and the lighter areas show the tissue substance. The leaflet on the right in this figure has been cut in a direction parallel to its flat surface so that the supporting cells are clearly shown; on the left are seen leaflets cut at right angles to their flat surfaces.

The more minute histological structure is shown in Text-fig. 5 and on Plates I and II. In the outer regions of the leaflets the typical respiratory arrangement is seen in which a simple respiratory membrane covers the blood vessels. The separate character of this membrane, as distinguished from the membrane which forms the blood channels, is indicated. At the base of the leaflets, however, the picture changes and the epithelium thickens into a type conforming more to stratified

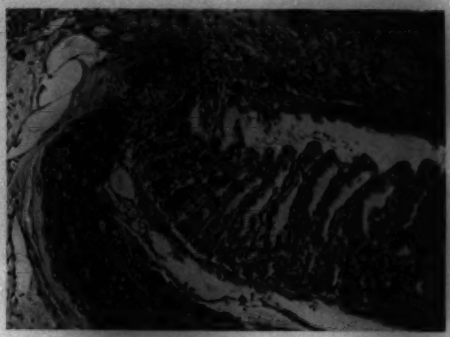


Fig. 1. Microphotograph (× 170) showing the position of the "chloride secreting" cells at the base of the leaflets (hæmalum and eosin).

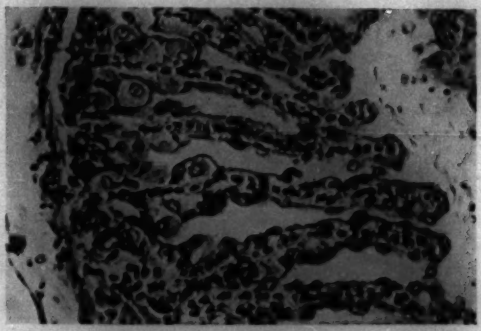


Fig. 2. Microphotograph (× 400) of leaflets with "chloride secreting" cells (hæmalum and eosin).

Microphotograph (× 880) of "chloride secreting" cells (iron hæmatoxylin, Heidenhain).

epithelium which in turn lies on a definite basement membrane of connective tissue. The latter is penetrated periodically by the blood vessels entering or leaving the leaflet.

In the proximal portions of the leaflets, particularly on the side nearest to the gill rays and therefore near to the blood vessels which

Text-fig. 5. Diagram showing the position of the "chloride secretory" cells. *a*, respiratory epithelium; *b*, "pilaster" cell; *c*, blood channel; *d*, "chloride secretory" cell.

bring blood to the leaflets, there are found large numbers of cells of a very different character from those forming the ordinary epithelium. The nucleus, instead of being more or less ovoid is almost spherical and is much larger; typically, it contains one nucleolus and a thin network of chromatin. Often it occupies a more or less central position in the cell, but sometimes the whole cell is slightly elongated and approaches the columnar form in which case the nucleus is situated towards the base of the cell.

The cytoplasm of these cells is finely granular and has a greater affinity for eosin than the rest of the epithelium. The cells are therefore conspicuous in sections stained with hæmalum and eosin or with Giemsa's azure eosin. The cells are typically ovoid in shape and may occupy the whole epithelial thickness, but they never, or only very rarely, appear to be more than one layer thick. They are more particularly confined to the region of the base of the leaflets, but isolated cells of the same character have been observed occasionally on the outer regions of the leaflets. In a single cross-section of the leaflets on the gills of the eel up to eight or ten of these cells may be found between a single pair of leaflets if the section has been cut in the neighbourhood of the gill ray. In other regions they are generally less numerous. The diameter of these cells is on the average 15μ, and the diameter of the nucleus about 6μ; both cell and nucleus are very markedly larger than the epithelial cells and their nuclei.

The presence of these cells of a definite secretory type on the leaflets of the gills and the experimentally found secretion of chloride by the gills at once suggested that the two may be intimately connected. Their position on the leaflets near the gill rays is interesting and may be significant. If these cells are doing a considerable amount of work it is probable that their demand for oxygen is high, so that they would tend to lower the oxygen saturation of the blood or fluid with which they are in contact. If they were situated elsewhere on the leaflets the blood would tend to leave the gills not fully saturated and by concentrating the cells around the afferent vessels this contingency is avoided; the blood can become saturated with oxygen after passing the secreting cells, and these can draw oxygen from the outside fluid as well as, or instead of, from the blood.

A number of other species were studied in order to see if there is any correlation between the presence or absence of these cells and the probable chloride-secreting capacity of the gills as inferred from the habitats of the various species. In the fishes so far studied none has shown as many of these specialized cells as occur in the eel, but most of them have exhibited cells of the same type although their form may vary somewhat. The findings with the various species have been condensed in Table I. The distinguishing criterion applied here to these cells was eosinophilia in very large cells with nearly spherical nuclei. As shown in the table, the character of the cells is somewhat variable, and it may be that there are forms intermediate between the true epithelial cells and those of the secretory type. This fact sometimes makes it difficult

to decide whether or not specialized secretory cells do occur, particularly in the fresh-water species where the presence of mucous cells between the leaflets adds a source of difficulty.

TABLE I.

Species	Number of cells	Description of cells and other notes
Anguilla vulgaris (the common eel)	Abundant between the leaflets, especially at their bases	Large round or ovoid cells, nearly spherical nucleus situated near the centre of the cell
Conger vulgaris (conger eel)	Two or three per section between each leaflet	Rather more columnar in shape than in the common eel, and the nucleus tends to be towards the base of the cell
Salmo salar (salmon)	Fairly numerous between the leaflets in adult	Smaller than in the common eel but well developed
Pleuronectes platessa (plaice)	Few except near the gill ray where they are well developed	Nuclei small and more condensed than in the eel
Cottus bubalis	A few	Rather small
Crenilabrus melops	Cells abundant	Well developed but nucleus rather small. Ordinary epithelium somewhat columnar
Scyliorhinus canicula (the rough dogfish)	None observed	Mucous cells abundant, also occurring between the leaflets
*Leuciscus rutilus** (roach)	Very few if any	Eosinophilic cells occur under the epithelium at the sides of the filaments
*Gobio fluviatilis** (gudgeon)	Cells fairly abundant, especially at the base of leaflets, in some sections, absent in others	Mucous cells occur among the eosinophilic cells
*Leuciscus vulgaris** (dace)	Very few	—
*Abramis blicca** (bream)	Fairly well developed at the base of the leaflets	Generally more tissue and mucous cells between the leaflets. The secretory cells do not have contact with both blood and external medium

* These are fresh-water teleosts.

In the eel and in several other species examined, the cells do not show any definite polarity, the nucleus being somewhat centrally placed in an ovoid cell. This may possibly be taken as an indication that the cells may be capable of working in either direction according to the osmotic concentrations. Incidentally there is a very striking histological resemblance between these cells and the oxyntic cells of the mammalian stomach whose function is the secretion of chloride, but in this case the cation is hydrogen ion, whereas in the eel the cation is sodium or potassium.

Mucous cells of the goblet cell type were also found on the gill filaments in all the species examined. In the marine teleosts and the eel these mucous cells were limited to the filament stalk itself, but in the fresh-water fishes and in the dogfish mucous cells occurred in abundance between and at the base of the respiratory leaflets.

Discussion.

It is rather surprising that the secretory cells described here were not reported earlier. It is true that they are generally limited to certain regions in the gill leaflets and may be entirely absent in some species. However, they are so clearly evident in properly fixed specimens that it seems probable that bad fixation may have been one reason why they were not noticed before.

These cells are present in large numbers in the eel; in animals of about 250 g. weight we estimated between three and six million cells in the gills of each eel. It is obvious that we have here a possible and even probable histological basis for the branchial chloride secretion. These cells, which we shall tentatively term chloride-secreting cells, are not only of an unmistakable secretory type and are not mucous cells, but they are located so as to be in close contact both with the blood and with the external medium. They are undoubtedly more pronounced in the eel than in the other species examined. The eel has an extraordinary power of osmotic regulation, and this may be ascribed largely to its proven ability to secrete chloride from the gills.

Evidence has been presented [Keys, 1931*b*; Smith, 1932; Keys, 1932] for the probability that the marine teleosts generally secrete chloride from the gills. The inference follows that the "chloride-secreting cells," if they really possess this function, should be present in all the marine teleosts; as has been shown, this expectation is realized and we found these cells in all the marine teleosts examined. In an elasmobranch, the dogfish, which is not dependent on a branchial secretory mechanism for its osmotic regulation [see Smith, 1931], we were unable to find chloride-secreting cells.

The salmon possesses "chloride-secreting cells" in a fairly well-developed condition, at least this was the case in the gills of a specimen which was captured while passing from sea water to fresh water prior to spawning. In a very young specimen taken from fresh water no trace of the cells could be found. This specimen, however, was fixed with formalin which has since been shown to be unsuitable for the

demonstration of this type of cell since it causes very pronounced shrinkage of the cytoplasm.

The presence of these cells in some of the fresh-water teleosts admittedly complicates the histological interpretation. However, it will be noted (Table I) that these cells are generally less abundant in the fresh-water teleosts than in the marine forms. They may, of course, be non-functional in the fresh-water species. Another possibility is that these cells are so organized that they do not necessarily secrete chloride out of the blood but that they always secrete in opposition to the concentration gradient. If this were the case they would tend, in the fresh-water fishes, to help maintain the salt content of the blood which is continually in danger of depletion in the fresh-water fishes.

A thorough survey of the histology of the gills of fishes from various habitats will be required before final conclusions can be drawn, but we feel justified in suggesting that the "chloride-secreting cells" described here are associated functionally with the branchial chloride secretion in the eel, at least, and probably in other marine teleosts.

From an evolutionary point of view it is perhaps interesting to note that the occurrence of mucous cells between the leaflets seems to be confined to the dogfish (the only elasmobranch examined) and the fresh-water species. They appear to have been lost in the essentially sea-water forms.

Summary.

The anatomy of the branchial circulation in the eel (*Anguilla vulgaris*) is described.

The histology of the gills of ten teleost species (including the eel) and of the dogfish (*Scyliorhinus canicula*) was studied. Cells of a secretory type were found to be present in large numbers in the gills of the eel and in smaller numbers in the gills of other teleosts; they were not found in the dogfish.

The possible correlation of these secretory cells with the chloride-secreting activity of the gills is discussed.

We are indebted to Mr J. Gray, Dr J. B. Bateman, Mr F. C. Stott and Mr F. P. Coyne for many of the specimens used in this work.

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THE DIURETIC ACTION OF ALCOHOL AND ITS RELATION TO PITUITRIN.

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That alcohol has a diuretic action is well known, but the way in which it produces its effect is not fully understood. Alcohol put into the circulation of a heart-lung-kidney preparation causes a cessation of urine flow according to Loewy and Bornstein [1927]. Hence it would seem probable that its action as a diuretic is extrarenal.

The experiments to be described represent an attempt to investigate the mechanism of alcohol diuresis. They fall into two series:

(I) Experiments on normal human subjects.

(II) Experiments on cats under amytal.

Part I. Experiments on human subjects.

Firstly, the diuretic effects of alcohol and certain of the more common diuretic drugs were compared. All experiments were carried out under comparable conditions; that is, at constant room temperature first thing in the morning, the subjects having no breakfast. In every experiment the volume of liquid taken was 300 c.c. at 29° C. Control experiments were done, water alone being drunk. The results in Table I show the comparative diuretic effect of alcohol (10 p.c. by volume), urea (5 p.c.), diuretin (theobromine sodium salicylate, 0·3 p.c.) and whey (made by clotting milk with rennin). It can be seen from these figures that alcohol has a very definite and rapid diuretic action. Adolph and Ericson [1926] considered that there are three types of diuresis: (1) water diuresis due to hydræmia, (2) saline diuresis, (3) drug diuresis. The rapidity of action of alcohol is like simple water diuresis. This latter is due to hydræmia and the resulting lowered osmotic pressure. It is antagonized by the antidiuretic hormone of the pituitary.

Experiments were carried out to show the relation of pituitrin to alcohol diuresis. Four different types of experiments were performed,

TABLE I.

No. and type of exp.	Urine flow per ½ hour before, c.c.	Urine flow in successive ½ hours after, c.c.				Total in 2 hours after, c.c.
Subject A						
31. Water, 300 c.c.	15	28	155	115	18	316
27. do.	19	50	216	136	12	414
24. do.	11	21	140	95	11	267
25. Alcohol, 300 c.c. 10 p.c.	17	19	134	214	148	515
28. do.	25	91	227	256	120	694
18. Urea, 300 c.c. 5 p.c.	15	Collected at the end of 2 hours				165
Glucose, 300 c.c. 25 p.c. (av. of 4 exps.)	16	do.				230
Glucose, 300 c.c. 25 p.c. and 10 p.c. alc. (av. of 6 exps.)	18	do.				444
Subject B						
24. Water, 300 c.c.	26	112	202	29	24	367
23. do.	18	56	233	52	10	351
25. Alcohol, 300 c.c. 10 p.c.	22	22	126	230	124	502
27. do.	16	68	235	265	43	611
10. Diuretin, 1 g. in 300 c.c. water	25	Collected at the end of 2 hours				365
Glucose 300 c.c. 10 p.c. (av. of 4 exps.)	21	do.				260
Glucose, 300 c.c. 25 p.c. and 10 p.c. alc. (av. of 6 exps.)	22	do.				485

all under the conditions mentioned above. The urine was collected every half-hour till the rate was constant, then an experiment of one of the following types was performed:

(A) 300 c.c. of tap water at 29° C. was drunk.

(B) 300 c.c. of tap water at 29° C. was drunk and an injection of 0·1 unit pituitrin was given.

(C) 300 c.c. of 10 p.c. alcohol at 29° C. was drunk.

(D) 300 c.c. of 10 p.c. alcohol was drunk and an injection of 0·1 unit pituitrin was given.

Subsequently the urine was collected at half-hourly intervals till the rate had returned to normal again. The results of these experiments are set out in Table II.

The pituitrin was Parke-Davis's posterior lobe extract (10 units per c.c.) diluted with sterile water 1 in 20 so that an injection of 0·2 c.c., which was the usual dose given, was equal to 0·1 unit. The small dose given in two experiments was half this (0·05 unit). The pituitrin was injected subcutaneously.

The results of these experiments show again the marked diuretic action of alcohol and also that this diuretic effect is able to overcome to a certain extent the antidiuretic effect of pituitrin. This point is brought

out by a comparison of Exps. 35 and 34, the small dose of pituitrin has a considerable effect on water diuresis. In Exp. 35 the time of maximum excretion is delayed by 1½ hours, the time of return to the normal rate by 1½ hours and the total excretion decreased by 110 c.c. In Exp. 34 the effect of the small dose is certainly weaker, the time of maximum excretion and the return to normal are only delayed by ½ hour and the total excretion only decreased by 27 c.c.: that is, the pituitrin is almost ineffective. It seems therefore that the alcohol antagonizes the antidiuretic action of pituitrin.

TABLE II.

No. and type of exp.	Urine vol. in c.c. per ½ hour: Before	Successive ½ hours after	Time of max. excretion (hours)	Time to return to normal (hours)	Total excretion till return to normal (c.c.)
Subject A					
31. A. Water diuresis	15	28, 155, 115, 18	1	2	316
27. do.	19	50, 216, 136, 12	1	2	414
30. B. Water, pituitary injection	16	14, 15, 13, 10, 19, 20, 19, 30, 61, 8	4½	5	209
25. C. Alcohol diuresis	17	19, 134, 214, 148, 6	1½	2	521
26. do.	25	91, 227, 256, 120, 21	1½	2	715
29. D. Alcohol, pituitary injection	13	13, 10, 11, 14, 75, 160, 58, 31, 21	3	4½	393
32. do.	11	12, 12, 11, 9, 85, 147, 128, 60, 28	3	4½	492
Subject B					
24. A. Water diuresis	26	112, 202, 29	1	1½	343
23. do.	18	56, 233, 52, 10	1	2	351
28. B. Water, pituitary injection	16	15, 18, 17, 20, 14, 17, 36, 38, 9	4	4½	184
22. do.	20	17, 25, 16, 10, 21, 39, 59, 25, 6	3½	4½	218
35*. do.	30	25, 16, 16, 14, 135, 22, 10	2½	3½	238
25. C. Alcohol diuresis	22	23, 126, 230, 124, 15	1½	2½	518
27. do.	16	68, 235, 265, 43, 17	1½	2½	628
33. D. Alcohol, pituitary injection	23	23, 15, 17, 22, 121, 42, 58, 15	3	4	313
34*. do.	20	30, 30, 126, 273, 69, 18	2	3	546

* Indicates where small dose of pituitrin was used, Exps. 34 and 35.

This could be brought about in several ways. We know from the work of Krogh that one of the most important functions of the posterior lobe of the pituitary gland is to maintain a normal degree of permeability of the capillaries. The absence of pituitrin in a perfusion liquid causes their permeability to increase. An injection of pituitrin into a normal animal probably makes the capillaries more impermeable. This may be the explanation of the antidiuretic effect of pituitrin in suppressing water diuresis. The blood is diluted to the same extent as in water diuresis [Priestley, 1921], but the urine is scanty because the water cannot be filtered through the glomerulus. Now the diuretic action of alcohol and

its antagonism to the antidiuretic action of pituitrin could be explained in several ways:

(1) That alcohol causes a greater hydræmia than water and thereby can overcome the impermeability due to pituitrin.

(2) That alcohol actually destroys or inhibits the secretion of the capillary substance of the pituitrin.

(3) That alcohol increases capillary permeability directly.

(4) That alcohol increases kidney volume and blood flow.

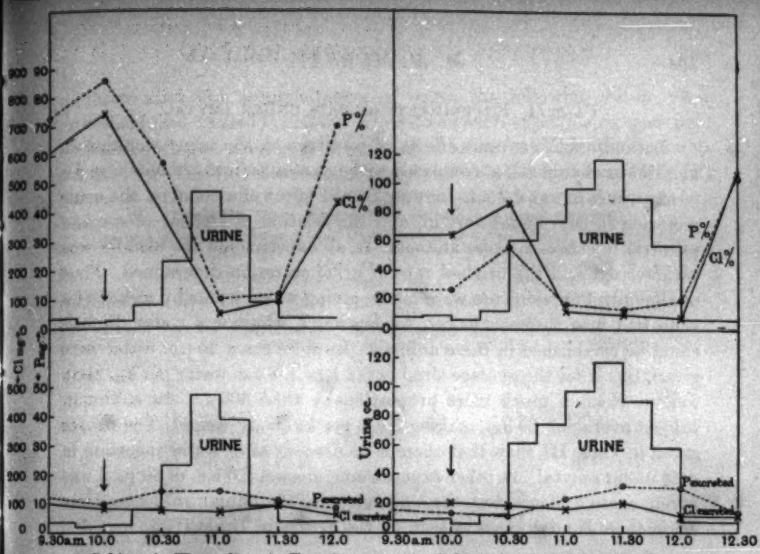
(5) That alcohol increases diffusion pressure in the glomerulus by increasing the blood-pressure, so that the decrease in permeability due to pituitrin is overcome.

Taking the last consideration first, the rise in blood-pressure from such a relatively small dose of alcohol is very slight or non-existent and, if this were the reason for alcohol diuresis, then a similar effect should have been obtained when alcohol was injected into anæsthetized cats in doses sufficient to give blood-pressure rise of about 10 mm. Hg. Here the alcohol did not produce diuresis. The experiments to show this are quoted in more detail later. Alcohol did not cause diuresis in the experimental animals, so the fourth point could not be tested.

Since the result of all the other three possibilities is ultimately the same, these effects cannot be dissociated. It is generally accepted that alcohol increases cell permeability. There is so far no good evidence that alcohol inhibits the secretion of the pituitary gland, but that alcohol and pituitary functions seem to be antagonistic was suggested by Edkins and Murray [1931]. With regard to the possible hydræmic effect of alcohol, attempts were made to investigate this by determining the osmotic pressure of the blood in these experiments, but the results were not consistent. Analyses of the urine were made in all cases, the results of four typical experiments are given in Figs. 1 and 2.

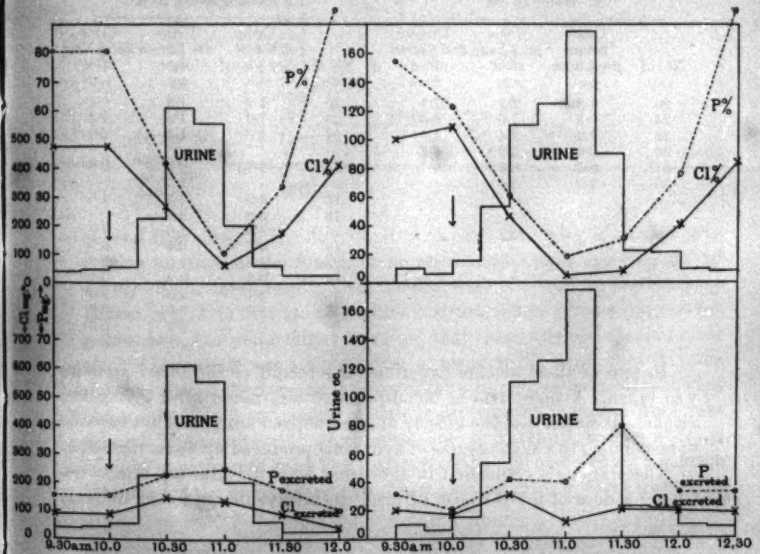
The variations in concentration seem to depend entirely on the urine volume, that is, alcohol causes an exaggerated water diuresis. The output of chloride and phosphate did not suffer much variation, though there was generally a small increase in phosphate output. In subject B there was a slight rise in chloride output and a more marked increase of phosphate; this was probably due to the very rapid rate of secretion.

The graphs of the urine excretion for alcohol experiments and simple water ingestions are very similar, suggesting a similar type of diuresis.



Subject A. Water diuresis, Exp. 24 — Subject A. Alcohol diuresis, Exp. 25

Fig. 1.



Subject B. Water diuresis, Exp. 23 — Subject B. Alcohol diuresis, Exp. 27

Fig. 2.

Part II. Experiments on cats under amytal.

Because of the reverse effects of pituitrin on the urine secretion in anæsthetized animals, as compared with unanæsthetized animals, a series of experiments was done to investigate the effect of alcohol on the urine secretion in cats under amytal, and the relation of alcohol effects and pituitrin injection in these animals. In all experiments the bladder was catheterized and the original rate of urine secretion determined. First of all control experiments were made, giving warm water by means of a tube tied into the œsophagus, to determine whether a water diuresis could be established in these animals. In most cases 20 c.c. water were given, this is for the average sized cat (2·7 kg.), 8 c.c. water per kg. body weight which is much more proportionally than 300 c.c. for a human subject averaging 60 kg., making 5 c.c. per kg. body weight. The figures given in Table III show that there is no diuresis after water ingestion in cats under amytal. In other experiments, alcohol (20 c.c. of 20 p.c.) was given, again a bigger dose than was given to the human subjects. Here again there is no diuresis as seen by the figures in Table III.

Table III. The secretion of urine in cats after administration of:

A. Water 20 c.c.				B. Alcohol 20 c.c. 20 p.c.			
No. of exp.	Urine before per ½ hour c.c.	Urine 1st ½ hour after c.c.	Urine 2nd ½ hour after c.c.	No. of exp.	Urine before per ½ hour c.c.	Urine 1st ½ hour after c.c.	Urine 2nd ½ hour after c.c.
20	1·2	1·3	1·1	20	1·2	1·2	0·4
23	0·8	1·0	0·45	23	1·0	1·3	1·4
24	1·6	1·4	1·0	24	1·9	1·9	2·0
26	0·6	1·0	0·8				
27	0·7	0·8	0·7	27	1·75	0·7	0·7
30	2·0	3·6					
				12	4·0	3·5	4·8
				13	3·8	3·6	
				16	2·7	2·0	2·0
				19	0·5	0·4	0·5
				22	1·6	1·0	0·9
				28	0·3	0·2	0·4
				31	2·7	2·3	2·0
				37	1·0	0·9	0·9
				38	0·3	0·3	0·3

In two of these alcohol experiments a record of the blood-pressure was taken. A slight rise in blood-pressure was noted after giving the alcohol. It seems that the kidney in anæsthetized animals is not capable of responding to a slight degree of hydræmia produced by water ingestion.

These animals responded in the usual way to pituitrin injections. Giving a dose of 0·5–0·75 c.c. of pituitrin intravenously to the animals

1 hour after the administration of water the following results were obtained (see Table IV, column A). Fifteen such experiments were performed, giving an average increase of urine in the first hour after injection of 13·4 c.c. This increase is to be compared with the figures in Table IV,

TABLE IV. Effect of pituitrin on diuresis in cats under amytal. Increase in urine excretion 1 hour after injection of 0·5 to 0·75 c.c. P.D. pituitrin.

	A. After 20 c.c. water ingestion		B. After ingestion of 20 c.c. 20 p.c. alcohol	
	A (i) 1st injection pituitrin	A (ii) 2nd injection pituitrin	B (i) 1st injection pituitrin	B (ii) 2nd injection pituitrin
No. of exp.	c.c.	c.c.	c.c.	c.c.
22			0·0	
12	15·0	—	2·6	
13	19·0	—	−2·3 (decrease)	
16	7·0	—	6·2	
20	13·5	—	1·0	
23	15·5	—	8·7	
24	23·0	—	14·2	
27	13·0	—	2·3	
Average (7)	15·0	—	4·1	
38	—	—	—	3·3
37	—	—	—	7·2
Average	—	—	—	5·0
8	4·7	5·1		
14	13·0	3·5		
15	11·0	12·0		
20	13·5	16·0		
32	9·0	4·8		
33	19·2	6·0		
35	8·9	12·2		
36	15·4	15·0		
Average (8)	11·8	9·4		
Average of all A (i) exps. (15)	13·4			

column B, where the injection of pituitrin after the giving of alcohol gave a urine increase in the first hour on an average (eight experiments) of 4·1 c.c. Since these results after alcohol were obtained on a second injection of puitrin into animals which had previously given a diuresis for a first dose, the point might be raised that there was less diuresis after alcohol because it was a second dose of pituitrin. To show that this was not the cause of the decrease in effect of the pituitrin, experiments showing the result of two successive injections after water ingestion were performed. The results of these are also given in Table IV, and an average of eight experiments gave 11·8 c.c. increase for the first and 9·3 c.c. urine increase for the second dose. In these cases no further water was given

before the second injection of pituitrin. Hence it is seen that the decreased effect of pituitrin after alcohol is not due to the fact that it is a second injection. It would appear therefore that whatever is the mechanism of pituitrin diuresis in these animals, it is antagonized by previous ingestion of alcohol. The concentration of alcohol in the blood in the animals was estimated in many cases, the usual concentration was 25 mg. per 100 c.c. blood.

Now whether these two sets of results:

(*a*) those on human subjects showing that alcohol is a potent diuretic and has an action antagonistic to the antidiuretic substance of pituitrin,

(*b*) those on cats in amytal anæsthesia showing that alcohol has no diuretic effect in these animals and that it antagonizes the diuretic action of pituitrin,

can be correlated is very difficult to say.

If alcohol antagonizes both antidiuretic and diuretic action, and, as will be shown in another communication, it also inhibits pituitrin hyperglycæmia, its effect seems to be very general. It also appears that alcohol, besides being able to antagonize these hormonic effects on blood sugar when pituitrin is injected, can also possibly antagonize the normal effect of the pituitry gland in these respects as well [Edkins and Murray].

Summary.

1. The diuretic action of alcohol on normal human subjects was compared with that of certain recognized diuretic substances and found to have a rapid and powerful diuretic action.

2. The diuretic effect of alcohol antagonized that of an injection of pituitrin in normal human subjects.

3. It is considered from the results that the effect of alcohol is an exaggerated water diuresis, not a specific effect on the kidneys.

4. In amytalized cats alcohol does not act as a diuretic.

5. Alcohol antagonized the diuretic effect of pituitrin injection in these cats.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

III. The relationship of blood phosphorus to liver glycogen and blood glucose in the decapitate cat.

By A. C. DE GRAFF, C. LOVATT EVANS and TOM VACEK[1].

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In their investigation of the carbohydrate metabolism of the fasting cat after decapitation, Evans, Tsai and Young [1931] concluded that the restitution of liver glycogen was greater than could be accounted for as occurring wholly from blood sugar or lactate, or both of these substances. Nevertheless it appeared to be significant that in its restoration the amount of liver glycogen tended to approach, but rarely if ever to exceed, that initially present in the liver at the beginning of the experiment, though that amount was often very different in different individuals. It was attractive to suppose that during decapitation the liver glycogen was converted into some intermediate substance which perhaps found its way temporarily into the circulation, to be ultimately returned in whole or in part to the liver and there reconverted into glycogen. This possibility was passed over by Evans, Tsai and Young in favour of the hypothesis of glyconeogenesis, but largely because there was at that time little to suggest the probable nature of such an intermediary.

Other investigators had felt similar difficulties with regard to the equating of glycogen lost by the liver or muscles [Corkill and Marks, 1930], though Simpson and Macleod [1928] found the discrepancy smaller for liver than for muscle.

The known relationship of phosphoric acid to carbohydrate metabolism, and the important work of Cori and Cori [1931] indicating that under various conditions muscle glycogen may be converted into hexose phosphoric acid, however, made it worth while studying the phosphorus changes of the blood in our decapitate animals.

Further, a formation of hexose monophosphate in glycogenolysis in the liver was indicated by Barrenscheen *et al.* [1930]. Apart from the

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possibility that the liver glycogen might be in part converted into hexose phosphate and retained as such in the liver pending its restoration to glycogen, there was the further possibility that hexose phosphate as well as glucose might find its way into the blood stream: in either case some change in the blood phosphates might be anticipated, and in the latter case it was just conceivable that the hexose phosphate of the blood might be considerable in amount, and since its reducing power is only about two-thirds that of glucose, the apparent inadequate loss of carbohydrate from the blood during glycogen formation might be due to the fact that part of what was estimated as glucose might be a quantity of hexose phosphate corresponding to an amount of glucose 50 p.c. greater.

It was found by Kay [1928] that the plasma never contains appreciable amounts of ester phosphorus. The red corpuscles, however, appear to contain hexose phosphate and glycerophosphate, and these in determinations by the Briggs' method would be estimated as organic phosphorus. According to Kay and Robison [1924] the corpuscles act as reservoirs for these esters which could diffuse from them as required into the plasma. We therefore used whole blood for the analyses, and estimated the inorganic and organic phosphate as a preliminary to a proper fractionation of the latter should the aggregate changes in its amount justify further enquiry. This in fact proved not to be the case.

Methods.

The methods used were in general those described by Evans, Tsai and Young, with the exception that the modified Shaffer-Hartmann method was employed for estimation of the glucose obtained from glycogen. An outline of this valuable method has been given by Murphy and Young [1932], by permission of Prof. Shaffer.

We determined the total phosphorus, inorganic phosphorus and glucose on the blood of cats before decapitation and at frequent intervals after decapitation. Liver samples were also taken for glycogen. The animals were all fasted 44–48 hours before the experiment.

The following modified Briggs' method was used for the phosphorus determinations in order to permit the use of reasonably small blood samples: 2 c.c. of blood were transferred immediately into 8 c.c. of a 1 p.c. solution of trichloracetic acid in a centrifuge tube. After shaking, 1 c.c. of 40 p.c. trichloracetic acid was quickly added and the total volume brought to 12 c.c. with distilled water. After thoroughly shaking, the tube was centrifuged for 10 min. and the supernatant fluid filtered. 5 c.c. of the filtrate were then used for the inorganic phosphorus and two 1 c.c. portions for duplicate total phosphorus determinations.

For inorganic phosphorus, to 5 c.c. of filtrate were added in succession 1·2 c.c. of 6*N* H_2SO_4: 2 c.c. of 5 p.c. ammonium molybdate, and 1 c.c. of hydroquinone and sodium sulphite solution: the volume was then made up to 10 c.c. and the solutions thoroughly mixed.

For total phosphorus, 1 c.c. of the filtrate was placed in a pyrex boiling tube; 1·2 c.c. of 6*N* H_2SO_4 added, and a glass ball placed on the mouth. The tube was then boiled on an

electric heater, as described by Stanford and Wheatley [1925] until the contents charred and dense fumes appeared. The tube was then cooled, 0·1 c.c. of Merck's perhydrol added, and the contents boiled again until the solution was clear and all the persulphate had been decomposed. After cooling, to the contents of the tube were added, with thorough mixing, 2 c.c. of ammonium molybdate and 1 c.c. of hydroquinone and sodium sulphite and distilled water to make the volume to 10 c.c.

For the standard 3 c.c. of KH_2PO_4 (1 c.c. = 0·02 mg. P) were made up in the same way as for the inorganic phosphorus determination. After half an hour the readings were made on a Klett microcolorimeter.

Results.

The blood phosphorus in the unanæsthetized cat.

We found as Kay did, that there is a wide range of normal blood phosphorus levels in the cat. In order to get some results for the unanæsthetized animal we performed cardiac punctures on a series of cats; samples can thus be obtained without causing pain or struggling. In four cats our figures were:

Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
9·7	10·6
9·3	10·3
7·5	10·6
5·0	9·2
Average 7·9	Average 10·2

With animals under ether anæsthesia the range from animal to animal was very much greater, as the following examples will show:

Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
4·8	9·6
8·3	11·8
5·3	6·1
4·1	18·1
8·9	7·6
7·1	11·8

The average for all our experiments for the initial blood samples under ether anæsthesia was 6·5 mg. for the inorganic phosphorus and 11·1 mg. for the organic phosphorus. Kay [1928] found a mean of 5·6 mg. for inorganic phosphorus in cats (presumably anæsthetized). His figures for the ester phosphorus of the corpuscles, 18–31 mg. (mean = 22), would also agree approximately with our figure for whole blood. In general there was a slightly lower value for inorganic phosphorus in the recently anæsthetized than in the unanæsthetized cat. This is in apparent disagreement with Martland and Robison [1924], who found that in rabbits ether anæsthesia and shock caused an increase in the inorganic

phosphate and also in the total phosphate, but after prolonged anæsthesia we obtained results similar to theirs. The greater range of variability in the anæsthetized animals might be due to differences in the depth of anæsthesia at the time when the samples were taken.

In the individual experiments, however, the blood phosphorus level changed very little as will be seen.

The effect of decapitation on the blood phosphorus. Although, on decapitation, we obtained the usual fall in liver glycogen with the concomitant rise of blood glucose, and after decapitation when the glycogen

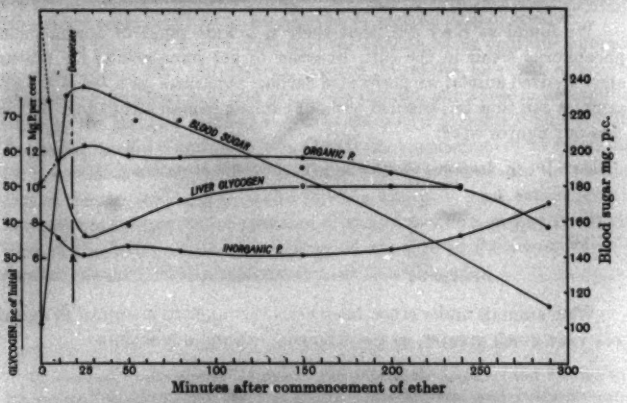

Fig. 1. Decapitate cat. Liver glycogen, blood sugar and inorganic and organic phosphate of blood. Mean curves from ten experiments.

again rose the glucose level fell, the blood phosphorus throughout showed little change. Such alteration as occurred, however, was fairly regular, and is best illustrated by Fig. 1, which represents the mean results of ten typical experiments.

The liver glycogen is expressed as percentage of the calculated original amount present at the commencement of etherization. The blood glucose and organic and inorganic phosphorus are shown as mg./100 c.c. of the whole blood.

It is seen that the inorganic phosphate, which is presumed to have been reduced by ether from about 8 mg./100 c.c. to about 7·1 at the end of 10 min., is further reduced after decapitation to about 6·1. The organic phosphorus shows a change in the opposite direction, from

11·5 mg. after 10 min. under ether, to about 12·3 after decapitation. The glycogen has fallen meanwhile from about 75 p.c. of the initial value to 36 p.c. The blood sugar changes are out of all proportion to the phosphate changes, the total rise of blood sugar from commencement of etherization to about 6 min. after decapitation being about 134 mg./100 c.c. There was a subsequent slight increase in the inorganic phosphate up to about 50 min., and afterwards a slow fall again. The organic phosphorus showed changes in the opposite direction of similar magnitude. During this time there was glycogen recovery.

We have little doubt that the fall of inorganic phosphate is to be explained on the lines indicated by the work of Cori and Cori, viz. a formation in muscle (and possibly in other tissues) of hexose phosphate from glycogen. Whether the rise in the organic phosphate of the blood is indirectly connected with this it is impossible to say. We frequently found, as shown in Fig. 1, that at the end of the experiment when the blood-pressure had fallen the inorganic phosphorus rose to above the initial value and the organic phosphorus fell below the initial level. These changes, however, need not be further considered here, because, owing to the large removals of blood for sampling, there was less complete recovery of glycogen in the later stages than usually occurs; in fact in some of the experiments averaged out in Fig. 1 a considerable fall of liver glycogen occurred in the later periods. Whatever may be the cause of these terminal changes, they probably have no connection with our present problem. It is evident, on the whole, that although considerable changes occur in both liver glycogen and blood sugar after decapitation, the blood phosphorus is not affected to an extent sufficient to justify its being considered as connected with the presence of notable stores of carbohydrate-phosphoric esters in the blood itself.

Effect of anæsthesia and repeated bleeding on the blood phosphorus level. In two experiments cats were kept under ether anæsthesia throughout, and only the usual sampling of liver and blood was carried out. As previously reported by Evans, Tsai and Young, the liver glycogen fell steadily, and the blood glucose first rose and then fell. The inorganic phosphorus showed an insignificant fall, but rose above the initial value again after 2 hours; the organic phosphorus after the first two samples fell considerably in one experiment, from 14 to 3 mg./100 c.c. in 1½ hours, but later rose again to 5·7. We have not further examined these delayed changes.

Relationship of adrenaline to the blood phosphorus in the decapitate cat. Barrenscheen, Eisler and Popper [1927] found in rabbits that as the

blood glucose rose after adrenaline injection the inorganic phosphorus of the blood was reduced. This has been confirmed by Cori and Cori [1932], who explained it as due to formation of hexose phosphate in muscle. We performed seven experiments on decapitate cats in which adrenaline was introduced slowly into the femoral vein at the time when recovery of liver glycogen had occurred after decapitation, and confirmed the observations of the workers mentioned. The curve of blood phosphorus was much the same as in the experiments in which decapitation alone was

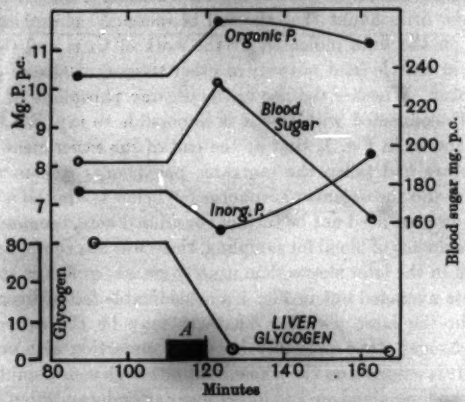

Fig. 2. Effect on blood phosphate and sugar and liver glycogen of infusion of 8 c.c. of 1 : 10,000 adrenaline into femoral vein of decapitated cat, during 10 min.

performed, *i.e.* there was a small fall in inorganic phosphorus and a corresponding rise in the organic phosphorus. The changes were quite small in amount, as shown in Fig. 2, which is illustrative of a typical experiment.

Effect of removal of both suprarenals on the blood phosphorus level. Removal of one suprarenal followed in 3 weeks by the removal of the other only caused a slight fall in the blood phosphorus as the following figures show:

	Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
Cardiac puncture before operation	9·3	10·3
Cardiac puncture after removal of both suprarenals	8·6	9·4

Further work on adrenalectomized animals was not attempted because of their feeble resistance to experimental procedures.

Effect of the injection of phosphate and glucose on the storage of liver glycogen. Two fasted cats were decapitated in the usual way and after recovery of liver glycogen we gave an injection of glucose and disodium hydrogen phosphate, in order to see whether there would be a further laying down of liver glycogen. In one experiment 5 g. of glucose with 1 g. of Na_2HPO_4 in 50 c.c. of distilled water were injected slowly into the femoral vein, and in the other experiment the same composition and amount of solution was allowed to run slowly into the splenic vein. In both instances we failed to cause any considerable rise in liver glycogen, although in the second experiment the liver glycogen did reach a slightly higher level than the initial level just before decapitation. The inorganic phosphorus rose steadily after the infusion of the glucose phosphate mixture, but the organic phosphate was not appreciably altered. Introduction of glucose into the duodenum in another experiment failed also to cause any storage of liver glycogen, nor was there any change in the blood phosphorus level contrary to the findings of Barrenscheen *et al.* [1927], McCullagh [1931] and others, who noted an increase in both organic and inorganic phosphorus in the blood when glucose was given *per os*.

Summary.

Decapitation of cats, or injection of adrenaline after decapitation and recovery, caused a fall of about 1 mg./100 c.c. in the inorganic phosphate of the blood and a similar rise in the organic phosphate. These changes are so small as to give no grounds for supposing that any appreciable amounts of carbo-hydrate-phosphoric esters can enter the blood during decapitation, or that such compounds in the blood form a source from which liver glycogen is formed.

The expenses of this investigation have been in part borne out of Rockefeller Foundation Funds (to T.V.), and in part out of a Grant from the Government Grants Committee of the Royal Society (to C.L.E.). Our best thanks are expressed for this assistance.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

IV. The effect of some anæsthetics.

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ERRATUM

Table I, Column A, 1st line
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method. Prof. Shaffer has kindly furnished us (through Prof. Lovatt Evans) with the unpublished details of the modifications of the original procedure, and with his permission we herewith include these, as we find the method as now modified to be entirely satisfactory and to give theoretical results with pure glucose.

Modified Shaffer-Hartmann reagent.

Na_2CO_3	25 g. per litre
$NaHCO_3$	20 " " "
Rochelle Salt	25 " " "
$CuSO_4$	7·5 " " "
KIO_3	100 to 300 c.c. of a solution 0·1 N as to I_2.

(We add 0·801 g. of KIO_3 per litre.)

5 c.c. of the neutralized glycogen hydrolysate are added to 5 c.c. of the above reagent and the mixture heated, in test-tubes (25 × 200 mm.) covered with glass bulbs, for 15 min. in a rapidly boiling water bath. After cooling in water to each is added 2 c.c. of a solution

[1] Bayliss-Starling Scholar. [2] Sharpey Scholar.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

IV. The effect of some anæsthetics.

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It has been shown [Evans, Tsai and Young, 1931] that the liver glycogen of the cat decreases under both ether and amytal anæsthesia. Since it would be desirable, if possible, to find an anæsthetic which would disturb the liver glycogen and blood sugar as little as possible, it was decided to carry out an investigation of the effect of some anæsthetics on the liver glycogen level in cats.

Methods.

The determination of liver glycogen was carried out in a manner similar to that described in a previous paper [Evans, Tsai and Young, 1931] with the following modifications:

After neutralization of the potash hydrolysate the glycogen was precipitated by adding alcohol up to 70 p.c.: the centrifuged precipitate, dissolved in 1 c.c. of water, was reprecipitated by adding 3 c.c. of alcohol, again centrifuged and hydrolysed. For the analysis of an aliquot portion of the neutralized hydrolysate we used the modified Shaffer-Hartmann method. Prof. Shaffer has kindly furnished us (through Prof. Lovatt Evans) with the unpublished details of the modifications of the original procedure, and with his permission we herewith include these, as we find the method as now modified to be entirely satisfactory and to give theoretical results with pure glucose.

Modified Shaffer-Hartmann reagent.

Na_2CO_3	25 g. per litre
$NaHCO_3$	20 " " "
Rochelle Salt	25 " " "
$CuSO_4$	7·5 " " "
KIO_3	100 to 300 c.c. of a solution 0·1 N as to I_2.

(We add 0·801 g. of KIO_3 per litre.)

5 c.c. of the neutralized glycogen hydrolysate are added to 5 c.c. of the above reagent and the mixture heated, in test-tubes (25 × 200 mm.) covered with glass bulbs, for 15 min. in a rapidly boiling water bath. After cooling in water to each is added 2 c.c. of a solution

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containing 2·5 p.c. of potassium iodide and 2·5 p.c. of potassium oxalate, and this is followed by 5 c.c. of $N H_2SO_4$. The tubes are kept covered by glass bulbs and, after shaking and standing for 5 to 10 min. to ensure complete solution and reoxidation of cuprous oxide and iodide, the mixture is titrated with 0·005 *N* thiosulphate. For calculating the results a curve may be constructed from the following data, supplied by Prof. Shaffer.

TABLE I.

Mg. glucose in 5 c.c.	Titration difference c.c. of 0·005 thiosulphate
2·00	18·20
1·75	15·95
1·00	9·00
0·50	4·48
0·10	0·85
0·05	0·42

Effect of sampling on glycogen content of lobes of the liver.

In accordance with the results described in the previous paper [Evans, Tsai and Young, 1931] the glycogen contents of different lobes of the liver have been considered to be comparable.

In order to determine whether the glycogen content of a lobe was significantly altered by the taking of samples, a number of simultaneous determinations were made on lobes, some of which had previously been sampled, and some of which had not, with the following results.

TABLE II. Glycogen content of used and unused liver lobes.

Exp.	Used lobe p.c.	Unused lobe p.c.
206	0·78	0·44
206	0·17	0·13
206	1·33	1·43
208	1·26	1·02
208	1·16	1·06
222	0·47	0·19
223	0·31	0·22
227	3·22	3·29
230	2·45	2·27
232	0·12	0·05
238	0·40	0·31
239	1·82	1·95
	Average 1·124	Average 1·030

Standard deviation = 0·087. Standard deviation = 0·094.

Unexpectedly it seems that the average of the used lobes is 0·094 p.c. greater than that of the unused lobes, *i.e.* nearly a 10 p.c. relative error.

Calculation of the standard deviation of the average for used and unused lobes suggests that this difference may or may not be significant, but whatever conclusion is drawn, it is certain that the glycogen content of used lobes is not lower, on the average, than that of unused lobes.

In a few cases one or more lobes appeared dark and bruised in consequence of handling or other injury. The glycogen content of such lobes was lower than that of the lobe which appeared quite normal, and histological examination showed that in the bruised sample there was stasis and congestion in the vessels and shrinkage and cytoplasmic cloudiness in the cells.

Blood sugar has been determined throughout by the method of Hagedorn and Jensen [1923]. Although Somogyi [1926] and others have shown that this method includes in the result what Benedict [1931] has termed "saccharoids," yet since we were interested principally in the relative blood glucose changes, we considered the method to be sufficient for our purpose.

At the beginning of each experiment we took a sample of normal blood from the ear for sugar determination. It was usually found possible to obtain such a sample without the slightest struggle while the cat was sitting peacefully on the table. In the few cases where struggling occurred the blood sugar level was always considerably above the average, and those results were not included in the average figures.

All the cats used in this investigation were fasted for 44–48 hours before the experiment.

Results.

Normal blood sugar of cats.

During the course of investigations in this laboratory blood samples from 89 unanæsthetized cats have been taken. The average blood sugar level of these animals was found to be 94·5 mg. p.c. (min. 62, max. 122).

In Table III are given the averages over various periods.

Table III. Average blood sugar for cats fasted 48 hours (Hagedorn and Jensen method).

Period	No. of cats	Blood sugar (mg. p.c.) Max.	Min.	Average
11. xi–10. xii. 31	22	120	79	98·8
11. xii. 31–26. i. 32	29	118	70	95·7
27. i–9. ii. 32	15	122	87	97·0
10. ii–25. iii. 32	23	121	62	86·9

During the period 9. ii–4. iii. 32, which included the coldest weather during the time of these experiments, the average blood sugar for 12 cats was 81·7 mg. p.c. The houses in which the animals are kept before experimentation are maintained thermostatically at a temperature of 60° F. The average duration of stay in the animal house is 4 days.

Whether the variation of average blood sugar for different periods shown in Table III is fortuitous, seasonal or dependent on temperature is, however, impossible to determine.

Effect of chloroform.

The induction of chloroform anæsthesia was effected by the use of an open mask, and continued after insertion of a tracheal T-cannula, by connecting this with a Woulffe's bottle containing chloroform, access of air being regulated by a screw clip on the side tube. In Fig. 1 are shown the average curves for liver glycogen and blood sugar obtained from three experiments. The liver in each case initially contained about 2 p.c. of glycogen, and the average curve has been extrapolated to 0 min.

It has been shown [Evans, Tsai and Young, 1931] that etherization for 50 min. reduces the liver glycogen to about 50 p.c. of its original level in cats. After 50 min. the liver glycogen content tended to rise slightly.

As was to be expected from its known toxic action on the liver, chloroform has a considerable effect on the glycogen content, reducing it to about one-quarter of the initial amount in 50 min. The blood sugar was also higher than that found under ether anæsthesia.

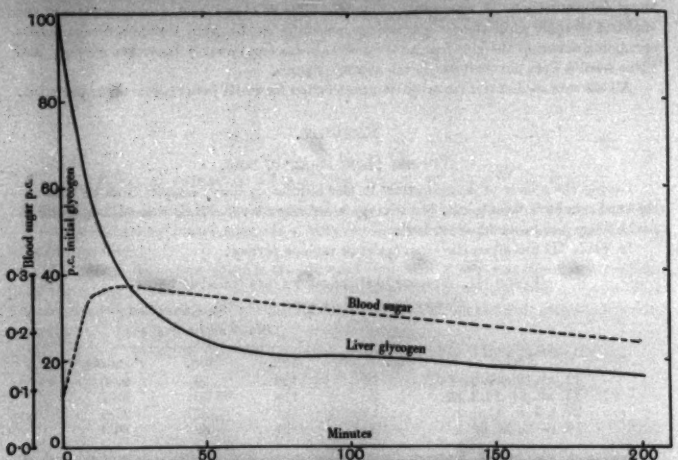

Fig. 1. Effect of chloroform anæsthesia on blood sugar and liver glycogen. Mean curves for three experiments. Chloroform begun at 0 min.

Effect of amytal.

Amytal (isoamyl ethyl barbituric acid) was one of the first anæsthetics shown to have little effect on the blood sugar level [Page, 1923; Edwards and Page, 1924], but in a previous paper [Evans, Tsai and Young, 1931] from this laboratory it was stated that amytal anæsthesia causes a considerable loss of liver glycogen in cats. This has been confirmed in the present investigation and the average blood sugar and liver glycogen curves for four experiments are given in Fig. 2.

We have found 100 mg. per kg. a generally satisfactory dose for intramuscular or intraperitoneal injection, but greater amounts (up to 160 mg. per kg.) are required subcutaneously.

Dann and Chambers [1932] have recently noted that the dose of amytal we used for cats (70–160 mg. per kg.) was considerably in excess of that required for dogs in their experiments (60 mg. per kg.), and consider that for dogs, at any rate, amytal anæsthesia is suitable for the study of carbohydrate metabolism.

We have invariably found with cats that if less than 70 mg. per kg. was injected intramuscularly satisfactory anæsthesia did not result even after 2 hours, at which time a further injection of 30 mg. per kg. produced surgical anæsthesia in 30 min. Our amytal was supplied by British Drug Houses, Ltd., and for injection was completely dissolved in 5–6 c.c. of dilute NaOH by warming to 50° C., the excess alkali being neutralised by dilute acetic acid, until the solution was faintly opalescent, one drop of alkali then being added to ensure complete solution.

Zerfas *et al.* [1928] find the intravenous anæsthetic dose for dogs to be 40–50 mg. per kg., whilst in man it is 20–25 mg. per kg., and they stress the fact that the *p*H of a 10 p.c. solution of the sodium salt of amytal in distilled water is 9·5–9·8, and claim that this *p*H gives a maximum anæsthetic effect in dogs with a minimum degree of toxicity. Lowering of the alkalinity of a 10 p.c. solution to *p*H 9·2–9·3, which causes some cloudiness, causes a striking loss in anæsthetic power, and a definite increase in toxicity.

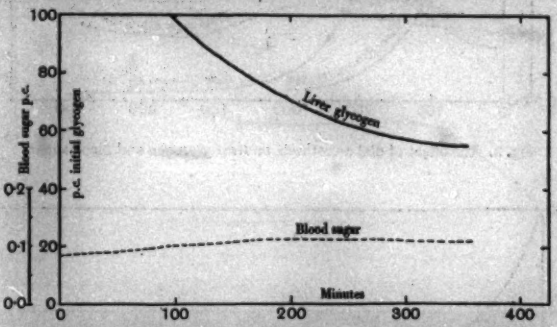

Fig. 2. Effect of amytal anæsthesia on liver glycogen and blood sugar. Mean of four experiments. Surgical anæsthesia about 100 min. after injection.

Page and Corylloss [1926] state that whereas 50 mg. per kg. intravenously is the anæsthetic dose for dogs, 15–20 mg. per kg. more is required for intraperitoneal injection. Fitch and Tatum [1932] find that the minimum lethal dose intraperitoneally for rabbits is 90 mg. per kg.

We are informed by Dr L. E. Bayliss (private communication) that he finds the amytal available in this laboratory to be less potent than that used by him at Harvard University (presumably Lilly's), of which he found 100 mg. per kg. to produce surgical anæsthesia in cats in 15 min. The amytal he used at Harvard was incompletely soluble in alkali, and after filtration was injected in alkaline solution, without neutralization.

We cannot explain this difference in potency of the amytal from these two sources.

Effect of dial.

Diallyl barbituric acid or dial (Society of Chemical Industry in Basle) was found to be effective in doses of 0·12 g. per kg. when dissolved in the same way as with amytal, surgical anæsthesia being induced in about an hour. The effect of dial in lowering liver glycogen and raising blood sugar

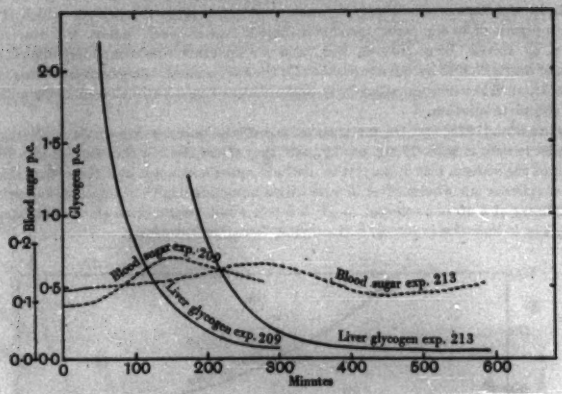

Fig. 3. The effect of dial anæsthesia on liver glycogen and blood sugar.

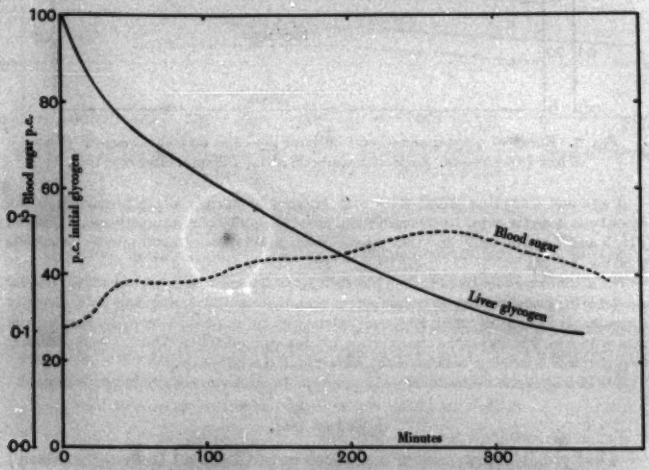

Fig. 4. Effect of luminal anæsthesia on liver glycogen and blood sugar. Mean of three experiments. Curve extrapolated to 100 p.c. liver glycogen at 0 min.

was found to be considerably greater than that of amytal, and the results are given in Fig. 3.

In spite of the fact that anæsthesia was very deep, the fall of liver glycogen content was extremely rapid, although the blood sugar was not raised above 175 mg.

Effect of luminal.

Sodium luminal (the sodium salt of phenyl ethyl barbituric acid (Bayer Products, Ltd.) in doses of 120–150 mg. per kg. in aqueous solution intraperitoneally was found to produce surgical anæsthesia in 20 min. In Fig. 4 are given the average liver glycogen and blood sugar curves for

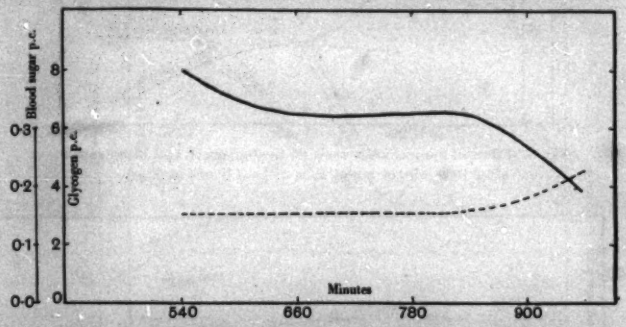

Fig. 5. Effect of luminal anæsthesia on liver glycogen and blood sugar after 9 hours of anæsthesia without liver sampling.

three experiments in which the initial liver sample was taken immediately after induction.

Arnell [1928] has stated that sodium luminal is without action on the sympathetic and the "parasympathetic" nervous systems. If this is so then it might be expected that if reflex effects were avoided this anæsthetic would have little effect on the glycogen stored in the liver.

This, however, was found not to be the case. The blood sugar progressively rose for 270 min. after which it fell, whilst the liver glycogen continuously diminished.

Figs. 5 and 6 give the results of two experiments in which the animal was allowed to remain anæsthetized during a long preliminary period before a liver sample was taken for analysis. By this means it was hoped that the liver glycogen would come to a steady state in which glycogenolysis was equal to glyconeogenesis.

During this preliminary period the temperature of the animal was carefully maintained at 37·5–38·5° C. on a hot plate, and water administered by a stomach tube if necessary. In one case blood sugar samples

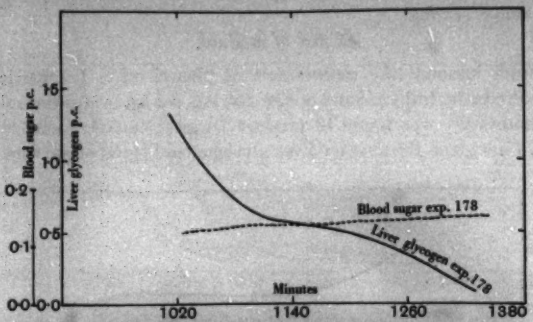

Fig. 6. Effect of luminal anæsthesia on liver glycogen and blood sugar, after 1000 min. of anæsthesia without liver sampling.

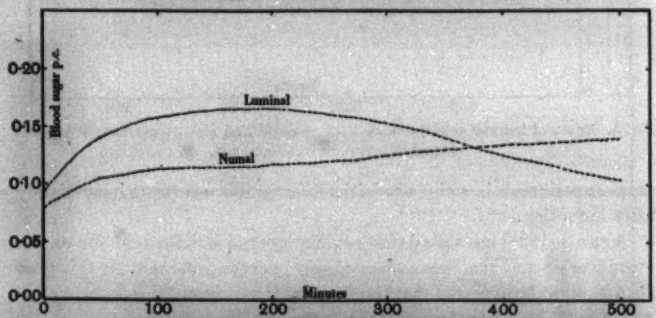

Fig. 7. Effect of luminal and numal anæsthesias on blood sugar. No liver samples taken. (Numal curve average of two experiments.)

were taken from the ear vein during this time, the curve being given in Fig. 7.

In the case of Fig. 5 the initial liver sample, which contained 8 p.c. glycogen, was taken after 9 hours of anæsthesia, and during the further period of 180 min., from 660 until 840 min., the liver glycogen and blood sugar showed little change.

In the experiment shown in Fig. 6, however, in which the initial liver sample was taken 17 hours after induction of anæsthesia, and contained 1·4 p.c. of glycogen, the liver glycogen content did not remain steady for any appreciable period, although from 1100 until 1220 min. the fall was very small.

Effect of numal.

Numal-Roche (the diallyl isopropryl barbiturate of diethylamine) is supplied by the makers in a 10 p.c. solution. Hoet and Ernould [1930] have found that this anæsthetic does not affect the blood sugar in rabbits,

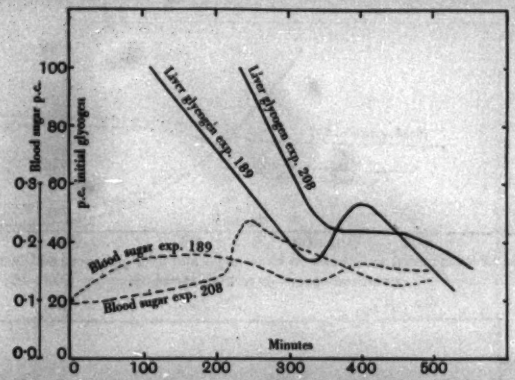

Fig. 8. Effect of numal anæsthesia on blood sugar and liver glycogen. Dose 0·7 c.c. per kg. intraperitoneally. Glycogen content of first sample in both cases approximately 3 p.c.

but Clark [1931] found that during surgical anæsthesia by numal in rabbits, and probably in cats, the hyperglycæmia following the intravenous injection of glucose is prolonged.

We have found (Fig. 7) that in an experiment in which blood samples were taken at intervals from the ear veins of two cats before and during induction of anæsthesia with numal, the average blood sugar steadily rose from 80 to 150 mg. in 500 min.

We have found the intraperitoneal injection of 0·5–0·8 c.c. per kg. of numal to be effective in producing surgical anæsthesia in about 100 min. in cats.

In Fig. 8 are given the results of experiments in which the liver samplings extended from 100 to 500 min. after the injection of numal,

whilst in Fig. 9 are given the results of two experiments in which a preliminary period of about 10 hours of anæsthesia preceded the liver sampling. In another experiment (Fig. 10) the first liver sample was

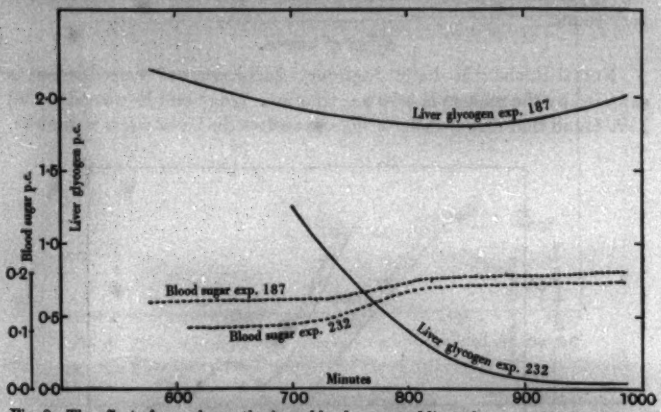

Fig. 9. The effect of numal anæsthesia on blood sugar and liver glycogen: 600–700 min. of anæsthesia previous to liver sampling.

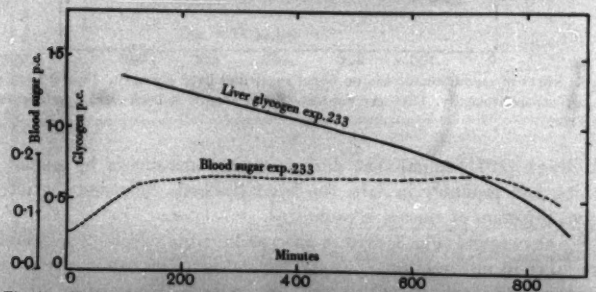

Fig. 10. Effect of numal anæsthesia on blood sugar and liver glycogen. First liver sample taken immediately after induction, the second 10 hours later.

taken immediately after anæsthesia had become satisfactory, the next being taken 10 hours after.

It seems from these results that the effect of numal anæsthesia on liver glycogen is very variable, though in all cases except one there was a

decided fall in the liver glycogen content, but it is difficult to explain the discrepancy between the glycogen curves shown in Fig. 9, as these two experiments were performed under similar conditions.

Effect of pernocton.

Pernocton is the sodium salt of sec. butyl bromallyl barbituric acid and is supplied by its makers (J. D. Riedel, Berlin) in 10 p.c. solution. The dose recommended is 1 c.c. per 12½–15 kg. body weight, but we found 0·5 c.c. injected intraperitoneally did not anæsthetize a cat weighing 2 kg. in 2 hours, after which time a further injection of 1 c.c. produced

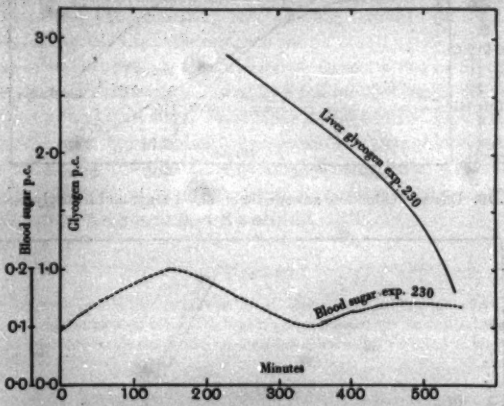

Fig. 11. The effect of pernocton anæsthesia on liver glycogen and blood sugar.

surgical anæsthesia after 1 hour. The effect on liver glycogen and blood sugar is shown in Fig. 11.

According to Dimitrijević [1930], in dogs the margin between surgical anæsthesia and death is narrow, and small rises of blood sugar occur under pernocton anæsthesia, though Matakas [1931] finds blood sugar and lactate are unaffected.

Effect of chloralose.

Vincent and Thompson [1928] found that chloralose raised the blood-pressure of cats and suggested that the secretion of adrenaline had been stimulated, but Tournade and Hermann [1928] could find no evidence for this theory.

We have found, in confirmation of Griffith [1923], that chloralose affects the blood sugar level very little, the theory of increased adrenaline

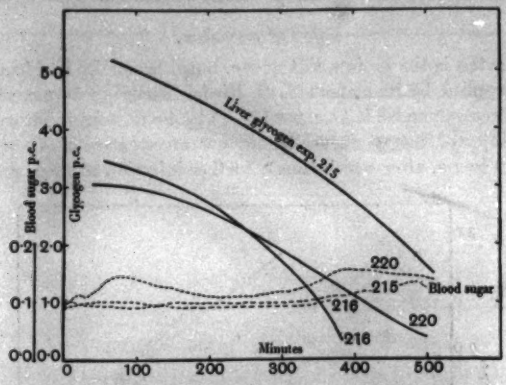

Fig. 12. Effect of chloralose anæsthesia on blood sugar and liver glycogen.

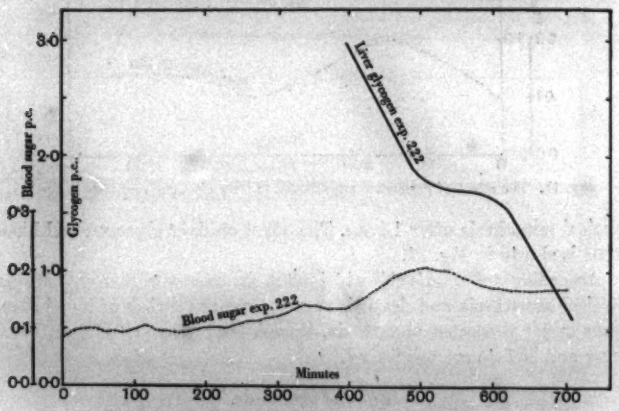

Fig. 13. Effect of chloralose anæsthesia on blood sugar and liver glycogen: 6½ hours of anæsthesia previous to liver sampling.

stimulation being thus not substantiated. Nevertheless, under the anæsthesia produced by subcutaneous injection of 0·1 g. per kg. body weight,

it was found, as it was by Bodo and Neuwirth [1931] in dogs, that the liver of cats rapidly lost glycogen (Fig. 12).

In Fig. 13 is given the result of one experiment in which a preliminary period of blood samples under chloralose anæsthesia preceded the taking of liver samples. In this cat the fall of glycogen was rapid at first followed by a period (500–620 min.) during which there was little fall, and again succeeded by another rapid fall. On the whole, however, the glycogen disappeared from the liver more quickly than in the short period experiments with chloralose.

In view of the very small change in blood sugar of cats anæsthetized with chloralose, the increasing rate of disappearance of liver glycogen with increasing time is rather surprising.

Magenta [1927] finds that chloralose causes a rise of blood sugar in the first 15 min., this being followed by a fall for 30–60 min. We found that the effect on the blood sugar was rather a slight fall at first with a slight rise after 5 hours. This eliminates the possibility that chloralose exerts its narcotic effect by the liberation of small quantities of either chloral or chloroform, as both of these anæsthetics produce a considerable hyperglycæmia [Steinmetzer and Swoboda, 1928].

Nembutal.

It has been suggested that nembutal (E. H. Spicer and Co.) has less effect on the liver than other anæsthetics used clinically, and we have carried out one experiment with this. As with other anæsthetics the liver glycogen fell continuously while the blood sugar rose somewhat.

Discussion.

Of the various anæsthetics we have tried, the blood sugar was least affected by chloralose, and, of the barbituric acid derivatives, least by amytal.

It is obvious that no anæsthetic that we have tried leaves the carbohydrate metabolism of the body undisturbed, under the conditions of our experiment, which involved the taking of successive samples of the liver. It would in fact appear very unlikely that any method of anæsthetization would eliminate the reflex glycogenolysis consequent upon operative procedures, without exerting some direct toxic action on the liver instead.

It seems, on the evidence of extrapolation, which is only approximate in view of the long period between induction and commencement of sampling, that the fall of liver glycogen, which is probably continuous from induction, is accelerated by the process of sampling.

It was also clear from experiments in which blood sugar determinations were made during a preliminary period of anæsthesia that the blood level was raised somewhat as soon as sampling of the liver commenced.

The rise in blood lactate and blood glucose, and the fall in alkali reserve [Fuss, 1931] under ether anæsthesia, together with the fall in liver glycogen, can in part be attributed to asphyxia, to a direct action of the anæsthetic on the liver, and to the liberation of adrenaline from the suprarenals [Macleod, 1926; Evans, Tsai and Young, *loc. cit.*]. Chloroform would appear to have a still more potent action of the same nature.

Brown and Garry [quoted by Clark, 1931] find that chloralose has less effect on the autonomic nervous system than any other anæsthetic they have tried. In experiments with cats under chloralose, Clark [1931] denervated the liver and tied off the suprarenals: he then found that there was a steady fall in blood sugar which was accelerated for a short time by the cutting of the right vagus. If his interpretation of this, *i.e.* that the vagus carries inhibitory fibres to the islets of Langerhans, be accepted, it seems possible from our experiments that chloralose may decrease the action of these inhibitory fibres, thus resulting in a liberation of insulin.

In those experiments in which the animal was kept under luminal or chloralose anæsthesia for a considerable period before the first liver sample was taken, there was, as shown by the curves (Figs. 5 and 13), a period during which the fall of liver glycogen was negligible.

During this period glycogenolysis was apparently either suppressed completely, or else was equal to the glycogen formation from protein fat or lactate: formation of glycogen from glucose is ruled out, in the case of luminal at least, by consideration of the blood sugar curves.

With numal anæsthesia in two cases, Fig. 8, Exp. 189, and Fig. 9, Exp. 187, the liver glycogen actually began to rise after the preliminary fall, the rise in each case being accompanied by a slight rise in blood sugar. It is clearly evident that glycogen formation could not have occurred from blood glucose, as this was rising, so that it must have taken place from some other source, possibly by glyconeogenesis, although evidence considered later tends to the view that amytal anæsthesia inhibits glyconeogenesis.

In most cases it is clearly impossible to account for the disappearance of liver glycogen by an increase in blood sugar. This is most clearly

seen in the action of chloralose, as the following detailed protocol shows:

Cat. ♀. 2·10 kg. Fasted 44 hours.

Time		Blood glucose p.c.	Liver glycogen p.c. (mean of two)
11.05	Blood sample from ear	0·101	—
11.06	Chloralose, 0·12 g./kg. intraperitoneally	—	—
11.27	Blood sample	0·098	—
11.58	Liver samples	—	3·48
11.59	Blood sample	0·104	—
3.07	Blood sample	0·097	—
3.14	Liver samples	—	2·21
5.00	Blood sample	0·111	—
5.01	Liver samples	—	0·88
5.50	Blood sample	0·113	—
5.52	Liver samples	—	0·19

Assuming that the blood sugar is in equilibrium with a volume of blood *plus* tissue fluids together equivalent to one-third of the body weight, the cat used in this experiment had 700 c.c. of fluid available for diffusion. At the beginning of the experiment the liver weighed 47 g., so that a fall of liver glycogen content from 3·48 p.c. at 11.58 a.m. to 2·21 p.c. at 3.14 p.m. should cause a rise of blood sugar of 0·085 p.c., *i.e.* glucose was being removed at the rate of 0·026 p.c. per hour (= 0·18 g. per hour).

In three experiments in which muscle and liver glycogens were determined at 60 and 350 min. after chloralose injection it was found that on the average the fall of liver glycogen during the period 100–350 min. was 0·65 p.c., whilst the muscle glycogen fell by 0·04 p.c.

The livers were weighed in each case, and the average loss of liver glycogen was calculated to be 0·46 g. Assuming that the musculature of the animal was equal in weight to one-half of the total weight of the animal the fall in muscle glycogen accounted for the disappearance of 0·93 g. of glycogen. During this period the blood sugar rose by an average of 20 mg. p.c., whilst the blood lactate fell by an average of 5 mg. p.c.

If the assumption is made that the volume of fluid in equilibrium with the blood sugar and lactate is equal to one-third of the body weight [Evans, Tsai and Young, 1931], then the rise of blood sugar and fall of lactate can together account for the disappearance of 0·17 g. of glycogen from the liver and muscles.

Thus of the 1·39 g. of glycogen which disappears from the glycogen stores of the body, only 0·17 g. can be accounted for in the blood, leaving a balance of 1·22 g. A similar result is obtained for experiments under amytal; presumably the 1·22 g. of glucose disappearing in 4 hours can be

more than accounted for by combustion in our experiments [Best, Dale, Hoet and Marks, 1926].

The question that remains, however, is, why do the glycogen stores of the anæsthetized cat suffer this depletion during a period that leaves the glycogen of an unanæsthetized cat materially unaffected?

The basal metabolic rate of amytalized animals differs little from the normal [Lee, 1928; Deuel, Chambers and Milhorat, 1926], and similar results would be expected for other anæsthetics, so that increased metabolism would not account for this difference.

The most reasonable assumption would seem to be that in the normal unanæsthetized animal the liver is continuously manufacturing glycogen, and that this "secretion" is inhibited by anæsthesia.

That amytal inhibits experimental hyperglycæmia has been confirmed by Olmsted and Giragossintz [1931] in the case of hyperglycæmia due to morphia and to asphyxia, and by Donhoffer and Macleod [1932] in the case of that due to asphyxia; Olmsted and Giragossintz [1931] suggest that amytal anæsthesia tends to inhibit glycogenolysis.

Donhoffer and Macleod [1932] have suggested that in the fasting rabbit decerebrated through the pons and with little liver glycogen, hyperglycæmia is due to stimulation of the glyconeogenic process in the liver by way of the parasympathetic nerves, and that this glyconeogenesis is inhibited by administration of atropine with section of both vagus nerves, and by amytal.

In view of recent evidence suggesting that amytal inhibits the parasympathetic [Weiss, 1929; Leib and Mulinos, 1929; Shafer, Underwood and Gaynor, 1930; Garry, 1930; Olmsted and Giragossintz, 1930; Donhoffer and Macleod, 1932] it would seem possible that the fall of liver glycogen under amytal anæsthesia is due to inhibition of the parasympathetic, and consequently of glyconeogenesis. Whether such a statement is true for other anæsthetics, in particular, for chloralose, cannot be decided on the available evidence.

However, as all anæsthetics that we have tried cause a decrease of liver glycogen under the conditions of our experiments, we must draw the conclusion that such conditions are unsuitable for the sudy of carbohydrate metabolism of the normal unanæsthetized animal.

Summary.

1. The effect of anæsthesia by chloroform, amytal, luminal, dial, numal, pernocton, chloralose on blood sugar and liver glycogen of the cat has been investigated.

2. In all the experiments in which liver samples were removed at intervals for analysis, it was found that the liver glycogen content fell throughout, or at some time.

3. Of the anæsthetics tried, on the average there was least fall of glycogen with amytal, whilst chloralose had least effect on the blood sugar.

4. In experiments in which the animal was anæsthetized some hours before the first liver sample was taken, the liver glycogen curve showed a "plateau," for the duration of which the fall of liver glycogen was small.

5. This can be considered to be evidence of the formation of glycogen in the liver, from non-carbohydrate sources.

6. It is concluded that experiments carried out under the above anæsthetics involving the taking of liver samples from experimental animals are unsatisfactory.

We wish to express our thanks to Prof. C. Lovatt Evans for much helpful advice and criticism, and to Mr C. A. N. Evans for technical assistance. We are also indebted to the Clayton Aniline Co. for samples of dial, to the Hoffman-la-Roche Co. for supplies of numal, and to Messrs Spicer for Nembutal.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

V. Some factors affecting liver glycogen recovery in the decapitate cat.

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Introduction.

The observation by Olmsted and Coulthard [1928] that the liver glycogen of the cat, lowered by decapitation, subsequently shows considerable recovery, was further investigated by Evans, Tsai and Young [1931 *b*] who pointed out that the decapitate animal during this recovery exhibits a rate of glycogen formation not to be obtained in any other laboratory preparation.

In continuance of that work we have further investigated some of the factors which may play a part in causing the restoration of liver glycogen in the decapitate cat.

The present paper deals principally with further consideration of the methods used, and with the influence of alterations in the amount and composition of blood supplied to the liver.

Methods.

The methods used were those described in the previous papers of Evans, Tsai and Young, and Murphy and Young [1932] with the following minor alterations:

(1) In order to ensure proper artificial respiration, the cats were ventilated, from just prior to decapitation, with a known volume of air (600–1000 c.c. per min., according to the weight of the animal). The double-action Schuster [1922] pump was generally used, but the Starling Ideal [1926] or the Olmsted and Taylor pump were employed on occasion.

(2) Carotid blood-pressures were taken at the times of blood sampling, and were usually found to remain constant at 50–80 mm. Hg, for several hours after decapitation. The fall to below 50 mm. after about 300 min. was often accompanied by a fall in liver glycogen. The average of 30 experiments showed that this deterioration began after the removal of 4·3 duplicate samples, at 260 min. after ether induction, and was sometimes accompanied by a slight terminal rise in blood sugar level.

[1] Bayliss-Starling Scholar. [2] Sharpey Scholar.

The procedure in the experiments was usually as follows: The cats were fasted for 44–48 hours. After taking a blood sample from an ear vein for blood glucose estimations, ether was given by an open mask, and all times were measured from the beginning of ether anæsthesia. Usual times for the ensuing procedures were:

0 min. Ether anæsthesia commenced.

4–6 min. Duplicate liver samples for glycogen. Tracheal cannulation.

12 min. Both carotids tied. Carotid blood sample.

17–25 min. Decapitation complete. Artificial respiration on. Ether off.

Then carotid samples and liver samples having been taken and blood-pressure records started, the experimental manipulation proper to the particular experiment was begun, and at suitable intervals subsequently carotid blood-pressure was noted, and blood and liver samples were taken.

After each removal of liver samples any scraps were also weighed, and at the end of the experiment a post-mortem examination of the carcase was made and the remaining liver weighed. The weight of the liver at each stage of the experiment could then be computed.

I. Further considerations regarding the methods.

(1) *Water content of liver and blood.*

It was just conceivable that the increase in liver glycogen in our preparations might be in part due to a loss of water from the liver. A number of liver samples were accordingly taken at various stages of the experiment, and dried to constant weight at 110° C. The moisture content estimated thus was found to be 69 p.c. $\pm$ 1 p.c., and under the conditions of the average experiment was found to be constant. This is in agreement with Bridge and Bridges [1931], who find in a series of rabbit livers with widely varying glycogen contents, a water content of 72 $\pm$ 4 p.c. Puckett and Wiley [1932] and MacKay and Bergman [1932] give similar values. We see no reason to differ from the conclusions of these various authors, or to suppose that in acute experiments such as our own the glycogen content is appreciably affected by water changes. The hæmoglobin content of the blood was also reasonably constant during the time of the experiments.

(2) *Fat content of the liver.*

In some preliminary experiments liver fat was estimated in successive samples, using the technique of Hynd and Rotter [1930], with a view to testing the theory, originally advanced by Rosenfeld [1903], of the reciprocal contents of fat and glycogen in the liver. In one experiment, shown below, there was a slight increase in "fat" (calculated from the fatty acid values with assumed mean molecular weight of linoleic acid – 280), but as there was no indication of any reciprocal adjustment between liver fat and glycogen contents in these experiments the subject was not further pursued.

Exp. 227. Cat. 2·25 kg. Fasted 44 hours.

	Liver		
Min.	Glycogen p.c.	Fat p.c.	Moisture p.c.
5	4·50	7·61	68·0
29 Decapitation	—	—	—
42	1·86	8·42	69·7
147	3·22	8·70	69·5

(3) *Method of liver sampling.*

The extended experience which has been gained with the methods employed for liver sampling has shown some important features which bear upon the interpretation of the results.

The experimental errors in the actual determination of glycogen in a given pair of duplicate samples are small and are chiefly due to the slightly greater degree of glycogenolysis in the second sample as compared with the first, and to experimental errors involved in the subsequent estimation. On the average these two sources of error only affect the results to the extent of 0·1 p.c. of glycogen.

The taking of two pairs of samples, at intervals, from the same lobe of the liver also does not result in the second samples giving too low values as compared with fresh lobes; indeed, as Murphy and Young [1932] have shown, the average for the second sample is usually very slightly raised as compared with intact lobes.

A more real source of error is the occasional variation in glycogen content from lobe to lobe, for although, as previously pointed out by Evans, Tsai and Young [1931*a*], on the average the lobes contain equal percentages of glycogen, still the occasional variations which are encountered make it important not to place too much reliance on the results of individual experiments. As would be expected, especially fluctuating results, with maintained high blood sugar and poor recovery of glycogen after decapitation, are liable to be encountered in those experiments which involve much manipulation of the abdominal viscera, including the liver itself: such manipulation or cooling of the viscera must therefore be reduced to a minimum.

It is our practice to weigh all the liver tissue removed at each stage, since the amount of liver remaining intact is of importance for two reasons. First, the removal of too much liver tissue might lead to portal obstruction, and this is particularly likely to happen if the lobes are sampled too far from their margins. The intestines were always carefully observed to ensure that there was no congestion. Secondly, as the liver is reduced by the taking of successive samples, an increase in its glycogen content becomes less and less significant to the balance-sheet of the whole body. An examination of 15 successive experiments taken at random showed that the livers had at the commencement of the experiments an average weight of 73·2 g. (2·8 p.c. of the body weight): the average weight of each sample (duplicate) removed, together with waste, was 2·48 g., *i.e.* in the taking of an average of nearly six samples some 14·7 g. of liver tissue (20·2 p.c. of the weight of the liver) were actually cut away.

But, in fact, more than this amount was put out of circulation by being enclosed between the bamboo strips or tapes used to check hæmorrhage; this amount varies with the number of lobes sampled and so with the number of strips or tapes simultaneously present on the liver. Estimating the tissue thus excluded, in the 15 experiments quoted above, the average loss by exclusion from the circulation of functional liver tissue amounts to about 3·3 g. per sample taken (2·43 min., 3·89 max.), which would mean that after six samples 19·8 g. of liver (27 p.c. of its weight) were out of commission. We do not consider it safe to go beyond this amount.

Now, supposing we start with a liver weighing 75 g. and containing 1·65 p.c. glycogen at the first sample, 0·66 p.c. after decapitation, 1·1 p.c. at the 100th minute and 1·58 p.c. at 300 min. (as in Evans, Tsai and Young's averages), and calculate the actual glycogen contents, with and without allowance for the reduction of liver tissue by three samples removing 3·3 g. each:

	Liver glycogen p.c.	Reduction of tissue: Not allowed for	Reduction of tissue: Allowed for
After 1st sample	1·65		
,, 2nd ,,	0·66	1·240 / 0·495 = 0·745 g. loss	1·185 / 0·450 = 0·735 g. loss
,, 3rd ,,	1·10	0·820 = 0·325 g. gain	0·710 = 0·260 g. gain
,, 4th ,,	1·58	1·180 = 0·360 g. gain	0·970 = 0·260 g. gain
		Total gain = 0·685 g.	Total gain = 0·520 g.

Similarly, whereas Evans, Tsai and Young [1931*b*, p. 94] calculate that between the 100th and 300th min. a 70 g. liver has gained 0·34 g. glycogen, recalculation shows that if two samples were taken, one at each of those times, the actual gain would be 0·32 g. The error due to loss by sampling does not appreciably affect their actual argument, but, as shown above, it becomes considerable when several successive samplings are considered. It will be evident that losses of total liver glycogen to the remainder of the body are also apparently increased, though to a smaller extent, by successive sampling.

(4) *Mode of expressing liver glycogen changes.*

The method used by Evans, Tsai and Young for expressing their results was to take a first sample under ether, and then calculate from this what was the approximate initial glycogen percentage before ether was given. This procedure involved the assumption that the livers of different animals lost the same fraction of their glycogen in the same time, while under ether; but it can be shown that if the animal has not been for many minutes under ether, the error is not likely to be great. The glycogen

contents throughout the experiment were then expressed as a percentage of this initial value. It was clear that this method left much to be desired, since the actual glycogen percentages in the livers of different 44-hour fasted cats varied greatly (from 0·33 to 10·0 p.c. in our joint experience), so that a loss or gain of a given percentage of the initial glycogen might represent widely differing amounts of glycogen. Careful inspection of all our results revealed the fact that often, though not invariably, the most rapid recovery was apparent in those experiments in which the initial

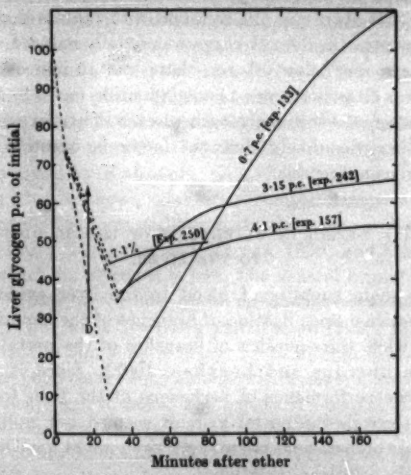

Fig. 1. Graph showing fall and recovery of liver glycogen of decapitate cats. Decapitation at the arrow marked *D*. Above each curve is shown the initial liver glycogen content.

concentration of glycogen was low, and in those experiments, moreover, a greater fraction of the liver glycogen was lost on decapitation. These facts are illustrated by Fig. 1 which gives the curve for four experiments taken at random and expressed in the manner described above.

It is clear from this example, as from most of our results, that the livers with the lower glycogen contents have lost a greater fraction of their glycogen on decapitation and have regained a still greater proportion of it on recovery: this is shown clearly by examination of the slopes of the recovery curves. Further examination shows that there was often a tendency for livers of similar size to recover total weights of glycogen of

about the same order during the same time in the early periods of the recovery. The weights of glycogen recovered were, however, not sufficiently near together to make it worth while expressing our results in that form for most of our purposes. We shall therefore adhere to the method previously employed, though it is important to bear in mind that the results of individual experiments cannot be properly compared with one another unless, among other identical conditions, their initial glycogen contents are similar.

It is perhaps not without significance in the present connection that Donhoffer and Macleod [1932] found with rabbits that after decerebration at the pons the liver glycogen was usually reduced, but that if the initial glycogen was below 0·7 p.c., there was an increase after decerebration; it was clear that some factor favouring recovery was present in those cases where the initial glycogen was low. Their experience, and our own, make it not unlikely that the factor in question is one which accelerates glyconeogenesis.

II. (1) Effect of alteration of the blood supply to the liver.

The two main supplies of blood to the liver evidently subserve different functions, since ligation of branches of the hepatic artery leads to necrosis, while the occlusion of branches of the portal vein leads to atrophy [Bainbridge and Leathes, 1907]. Since it is a common practice in the performance of perfusions of the liver to circulate the blood only through the portal vein, it seemed not unlikely that the failure of those preparations to store glycogen might have been due to the omission of the arterial circulation.

(*a*) *Ligation of the hepatic artery.*

Somewhat different estimates have been given by various investigators for the relative contributions to the hepatic blood supply of the hepatic artery and portal vein. According to Burton-Opitz [1911], and Macleod and Pearce [1914], about 30 p.c. of the total flow through the liver, in the dog, is derived from its arterial supply. Barcroft and Shore [1912], for the cat, give figures ranging from 13 to 58 p.c., the higher figure being for fed animals: they believed that most of the oxygen supply is obtained from the hepatic artery blood. Grab, Janssen and Rein [1929], for the dog, give the smaller figure of about 18–25 p.c. for the arterial fraction, while Bauer, Dale, Poulsson and Richards

[1932] in artificial perfusion found 25–30 p.c. for cat and dog. It would seem certain, from these results; that at least one-quarter of the blood supply to the liver is conveyed by the hepatic arteries.

It is well established that, in man, complete occlusion of the hepatic artery supply is not incompatible with life. In other animals too, many instances are recorded of recovery after ligation of the hepatic artery; an analysis of such cases is given in the papers by Segall [1923] and Cameron and Mayes [1930]. The explanation of these results is that ligation of the hepatic artery, even if complete, does not necessarily connote total deprivation of arterial blood supply to the liver, since, as pointed out by Haberer [1906], Segall [1923] and others, there are anastomotic connections of variable extent between the branches of the hepatic artery and those of the diaphragmatic arteries.

In the cat, the effects of complete occlusion of the hepatic artery are usually severe, though exceptions attributable to adequate collateral circulation were found by Haberer.

It has been clearly shown that after complete deprivation of its arterial blood supply the liver undergoes necrotic changes [Bainbridge and Leathes, 1907; Cameron and Mayes, 1930; and others], at an early stage of which its glycogen is lost. The general importance of the hepatic artery supply in carbohydrate metabolism was indicated by Collens [1925]. Collens, Shelling and Byron [1926, 1927] further found that ligation of the hepatic artery in dogs often caused death in 15–50 hours in hypoglycæmic convulsions, with complete loss of glycogen from the liver, heart and muscles. The period of survival after ligation they found to depend upon the glycogen content of the body. Winternitz [1911] and others describe the pathological effects as being different for different animals, and obtain as a rule little effect on the dog. It is probable that some earlier workers, not quoted here, did not get complete deprivation of arterial supply in their experiments.

In view of the facts just mentioned, it might be expected that on ligation of the hepatic artery in the decapitate cat there would be a failure of the normal recovery of glycogen if the latter was in any way dependent, as the ultimate survival of the liver tissue is dependent, on the provision of a proper supply of arterial blood. As will be shown, this expectation was not realized, for we found the recovery to occur fairly well in spite of the severity of the operative handling.

The arterial supply was first studied in injected preparations, from one of which Fig. 2 was sketched. There are considerable individual variations.

The hepatic arterial supply was cut off by two ligatures applied, one between the right gastric artery and the root of the hepatic artery proper (*b*, Fig. 2), and one to the gastro-duodenal artery (*a*, Fig. 2). This is necessary because the hepatic artery, after giving off the gastro-duodenal, is almost invariably split up into three or four lobar branches, the

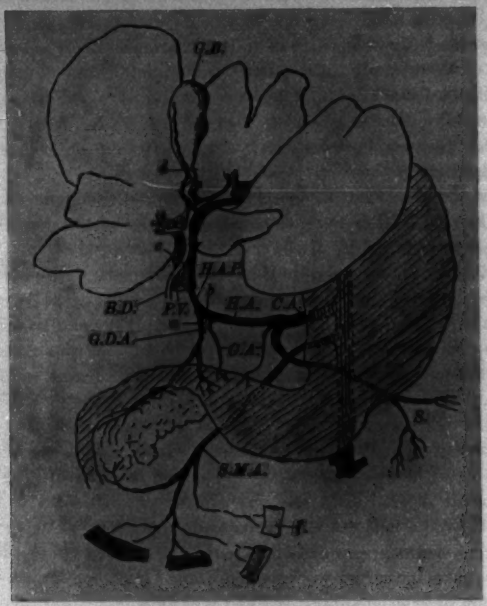

Fig. 2. Arterial supply to the cat's liver. *C.A.* cœliac axis; *G.A.* gastric arteries; *H.A.* hepatic artery; *H.A.P.* hepatic artery proper; *S.* splenic artery; *G.D.A.* gastroduodenal artery; *S.M.A.* superior mesenteric artery; *B.D.* bile duct; *P.V.* portal vein; *a* and *b*, position of ligatures on arteries; *c* and *d*, ligatures on bile ducts.

posterior of which are inaccessible for ligation as they pass medially and backwards to the left and behind the portal vein, which has the bile duct lying above its right border; further, because the collateral circulation by the gastro-duodenal artery is known to be adequate to maintain the liver. The effectiveness of the ligation was confirmed at post-mortem. It was complete in three experiments and incomplete in one.

The visible effects of the ligation during the course of the experiments, which lasted up to 7 hours, were not very striking—the liver became darker in colour, and showed congested patches, while the gall bladder, though not distended, was generally much lighter in colour than the normal, a greenish yellow in the most extreme cases—and there was some degree of diffusion of bile from the gall bladder on to adjacent parts of the liver.

In one experiment the animal was killed 6¼ hours after the ligation. Some portions of the liver appeared very congested in patches, with appearance of early necrosis; the greater part of the tissue appeared to be only a little more solid than the normal. Portions fixed in Bouin's fluid confirmed the naked eye appearance. The normal-looking portion had a

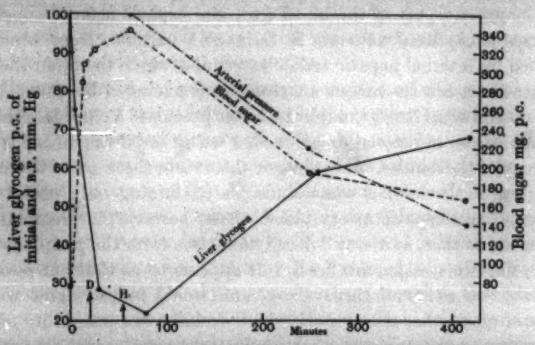

Fig. 3. Cat. Calculated initial liver glycogen concentration = 5·8 p.c. Decapitation at *D*. Glycogen at 30 min. = 1·62 p.c. Hepatic arteries ligatured at *H*. Glycogen at 79 min. = 1·29 p.c.: at 259 min. = 3·44 p.c.: at 420 min. = 3·94 p.c.

normal structure, the congested portion showed much the same appearance as described by Cameron and Mayes. There was great engorgement of all the sinusoids and of the branches of the portal vein and hepatic artery. In the portal vein blood were many mononuclear cells, looking like large detached Kupffer cells. The cytoplasm of the cells was much more cloudy than in the normal patches and showed vacuolation in some areas near to the intralobular veins.

The result of one of the experiments with complete ligation is shown in Fig. 3. The other three gave similar results, though in one of them the rise, which was good up to about 200 min. was then replaced by a fall.

In two experiments the blood sugar reached a high level after the ligation of the arteries (0·333 and 0·343 p.c.), and in three experiments out of the four, fell rather less quickly than usual, and even at about 350 min. still had, for the four experiments, a mean value of 0·122 p.c., whereas in normal conditions the amount would be below 0·1 p.c.

It appears from these results that, whatever may be the function of the arterial supply to the liver, its presence is not a basal necessity for glycogen formation, since it can be dispensed with for several hours.

In attempting to interpret this unexpected result some reference is necessary to the current views regarding the relationship between hepatic and portal circulations, an adequate summary of which is given by Cameron and Mayes [1930], Olds and Stafford [1930] and McMichael [1932].

The investigations of Cameron and Mayes lend support to the view that the greater part of the blood from the hepatic arterial supply first traverses a capillary network in Glisson's capsule, from which it is collected by internal hepatic radicular venules which then join the portal stream: direct arterio-venous anastomosis is excluded by them, as also by Olds and Stafford, though the latter postulate a considerable direct communication of arterial capillaries with the portal sinusoids at the periphery of the lobules. According to their view, there are at the extreme periphery of the lobules some sinusoids containing only portal blood, and some only hepatic artery blood: these, however, intercommunicate further on, so that, at about 3–7 cell diameters from the periphery of the lobule, the two streams mix freely. It appears to us that our results are explicable on either of these views, and would indeed agree with the results of most other recent work which excludes the possibility of direct arterio-venous anastomoses. Our results, moreover, seem to point to the conclusion that, since the glycogenic power of the liver is not immediately impaired by reduction in its oxygen supply, the subsequent loss of glycogen which is evident about 12 hours after occlusion of the arterial supply may be secondary to changes in the circulation in Glisson's capsule. It is possible, however, that the deprivation of oxygen may not really be so great as would at first appear from the statements regarding the participation of the hepatic artery in the total blood flow through the liver. It was shown by McMichael that clamping of the hepatic artery only caused a slight fall in portal pressure, and Bauer *et al.* showed that it was accompanied by an increased inflow by the portal vein and that there was a measure of reciprocity between the two flows, so that the outflow, when both supplies were available, was less than that calculated from the sum of the two isolated supplies. Under the conditions of our experiments, moreover, this reciprocal effect would be likely to be enhanced, since occlusion of the hepatic and gastro-duodenal arteries would probably cause blood to be directed to those abdominal organs

which drain into the portal veins. Lastly, there might still be some arterial supply from the collateral circulation with diaphragmatic arteries, though this would probably be a small one.

(*b*) *Reduction of the portal inflow.*

The whole blood flow through the liver is certainly large, and is estimated by Grab, Janssen and Rein [1929] at from 50 to 60 p.c. of the total return by the inferior vena cava. Even if we accept a high figure for the hepatic artery inflow, we are left with a portal inflow of the order of 40 p.c. of the inferior cava current. As it was not proper, for obvious reasons, to attempt to reduce the portal inflow by direct constriction of the portal vein, we effected it by tying off various arteries of supply to the splanchnic area, or by partial evisceration.

In the experiments of arterial ligation we tied off either the superior mesenteric artery or the cœliac axis, or both, causing as little damage as possible to the periarterial nerve plexuses. When either vessel was tied off, there was only an evanescent duskiness of the intestines, since anastomotic connections are very free. When the superior mesenteric artery was tied off, the liver received its arterial supply by the normal channels, as well as the portal return from the cœliac axis and inferior mesenteric arteries. This procedure was shown by McMichael to produce a sudden and considerable fall in portal pressure, much greater than that produced by clamping the hepatic arteries, though it is uncertain to what extent the reciprocal adjustment of circulations, referred to above, may compensate for the reduced portal inflow by a greater hepatic artery supply. However, the fact remains that in two experiments of this type, both showed a remarkably good return of liver glycogen, at least for a time. One of these experiments is shown in Fig. 4.

The cœliac axis was tied close to the aorta in one experiment. In this the liver still received an indirect arterial supply from the superior mesenteric artery by way of the anastomotic gastro-duodenal artery (*vide* Fig. 2) as well as the normal or even enhanced portal return from the territory fed by the superior mesenteric artery and the small inferior mesenteric tributary. In this experiment the return of glycogen was also very prompt and large, though it fell away later as was the rule in many control experiments. This experiment is shown in Fig. 5.

When the cœliac axis and the superior mesenteric arteries were both tied, leaving only the insignificant portal return from the inferior mesenteric artery to supply the liver, the liver glycogen, as might be anticipated, fell off rapidly and showed no return (Fig. 6). As the blood sugar also

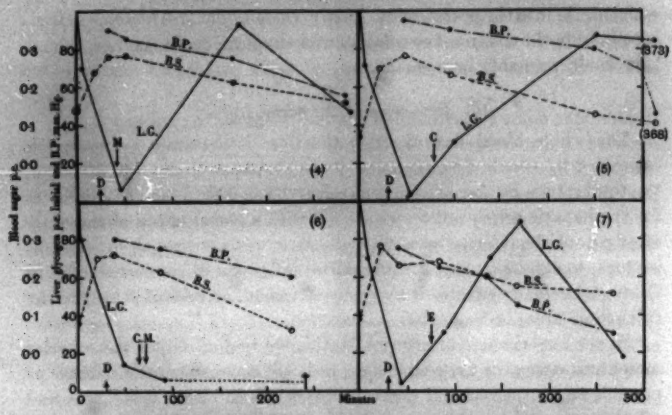

Fig. 4. Cat. 2·45 kg. Fasted 44 hours. Decapitation at 24 min. followed by ligation of superior mesenteric artery (M) at 41 min. Calculated initial liver glycogen = 2·12 p.c. Percentages at other times (ether at 0) were:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
5	1·59	171	1·98
45	0·14	290	1·00

Fig. 5. Cat. 3·0 kg. Fasted 48 hours. Decapitation at 30 min. followed by ligation of cœliac axis (C) at 78 min. Calculated initial liver glycogen = 2·13 p.c. Ether at 0 min. Glycogen concentrations at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
6	1·53	250	1·84
53	0·06	373	1·60
101	0·66		

Fig. 6. Cat. 3·4 kg. Fasted 44 hours. Decapitated at 30 min. Ligation of cœliac axis and superior mesenteric artery at 64–71 min. Calculated initial liver glycogen = 1·48 p.c. Glycogen concentrations at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
3	1·18	92	0·09
49	0·21	234	(?)0·00

The animal died between 234th and 292nd min.

Fig. 7. Cat. 3·3 kg. Fasted 44 hours. Decapitated at 29 min. Partial evisceration (see text), completed (E) at 75 min. Calculated initial liver glycogen = 1·58 p.c. Glycogen at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
8	1·11	139	1·11
43	0·06	171	1·41
89	0·48	269 and 279	0·22 and 0·31

fell quite rapidly, it was probable that we were dealing, in fact, with a virtually completely eviscerated preparation.

In the partial evisceration experiment we removed the spleen, and the intestine with its mesentery from the third part of the duodenum to the rectum, and ligated the superior mesenteric artery. There thus remained the normal (or enhanced) hepatic arterial supply and the venous return from stomach, pancreas and duodenum. Here again there was excellent recovery (Fig. 7).

On the whole these experiments with restriction of the portal flow show that there is considerable latitude in the vascular requirements of the liver. It is tempting, since the recoveries of glycogen were at first all rather above the average performance, to suppose that the restriction of venous return has, by allowing of a larger relative arterial supply, or in some other way, actually augmented the rate of recovery, However, such a conclusion is hardly justifiable without further experiments to support it.

In one experiment, after decapitation, we made a "reduced preparation" by tying off the aorta just above the origin of the inferior mesenteric artery, and the inferior vena cava just below the entrance of the renal veins. This was intended as the preliminary to an attempted visceral preparation similar to that described by Markowitz and Essex [1930], which consists of heart, lungs, alimentary canal, pancreas and liver. The preparation gave fairly good recovery up to 155 min., but the arterial pressure then rapidly fell and the liver glycogen was quickly lost again.

We also got fair recovery in another experiment in which we effected still more reduction by tying off both axillary arteries at their first part, and the aorta just below the superior mesenteric, the corresponding veins not being ligated: in this instance the arterial pressure and liver glycogen were well sustained until about 170 min. These experiments were not further pursued, however, because, although it was evident that a transfusion of some kind was desirable in order to maintain proper circulatory conditions, we had not at that time much knowledge of the effect of defibrinated blood, or of anti-coagulant materials, on the recovery process. These points will be discussed later.

(2) Effect of occlusion of the bile ducts.

It was shown by Bernhard [1931] that when the common bile duct is ligated in rats the animals survive longer when given glucose injections. This he attributes to deposition of liver glycogen. We carried out three experiments in which, after decapitation, the common bile duct and cystic duct were tied off (Fig. 2 *c* and *d*). All gave similar results. By the

end of the experiments (about 7 hours in two cases; death at 4 hours in one) there was considerable distension of the hepatic ducts. The following is a protocol of the most typical experiment:

Exp. 201. Cat ♂. 4·3 kg. Fasted 48 hours.

Time min.	Procedure	Arterial pressure mm. Hg	Blood sugar mg./100 c.c.	Liver glycogen actual p.c. (mean of 2)	Liver glycogen p.c. of initial
−2	Ear puncture	—	95	5·16 (calc.)	100
0	Ether given	—	—	—	100
5		—	—	3·88	75
9	Respiration stopped. Gave A.R.	—	—	—	—
13		—	273	—	—
18	Decapitation completed. A.R. by Schuster pump, 750 c.c. at 19 strokes, per min.	—	—	—	—
25		120	302	—	—
42	Common and cystic ducts ligatured	—	—	—	—
44		—	—	0·71	14
47		—	—	0·72	14
52		125	261	—	—
186		95	269	—	—
190		—	—	2·23	43
286		85	184	—	—
293	37 c.c. urine drawn (trace of bile?)				
295		—	—	3·08	59
376		55	143	—	—
426	(4 liver samples)	—	—	2·05	40
437		35	118	—	—
440	Killed. P.M.				

Wt. of liver left = 59 g.
Initial wt. of liver = 79 g.

The experiments all showed that for some hours after ligation of the bile ducts recovery of liver glycogen is still possible. The recovery in the experiment given above is perhaps rather on the low side, even when the rather high initial glycogen content is considered. In another experiment with the low initial glycogen concentration of 0·38 p.c. the recovery was, however, well above the average and reached 190 p.c. of the initial value in 168 min., though this was not sustained. On the whole, we may say that for the first 2 or 3 hours occlusion of the bile ducts makes little difference to glycogen storage in the liver of the decapitate cat.

III. Effect of alterations in the amount or composition of the circulatory blood.

It was found by Evans, Tsai and Young [1931 *b*] that, when compared with the rapid rate of spontaneous recovery of glycogen after decapitation, the effect of infusion or other exhibition of glucose had a

relatively slight effect on the glycogen formation. This was surprising, because the readiest assumption was that the glycogen which accumulated so rapidly in the liver after decapitation was formed principally from the blood sugar, which was always high just after decapitation, and which, in fact, did fall during recovery. They not unnaturally expected that if this were the case, the addition to the blood stream of a considerable amount of glucose would not only greatly accelerate the speed at which glycogen was laid down, but would lead to the attainment of concentrations of liver glycogen considerably beyond those present to begin with. Since neither of these things happened it was concluded that either the circulating precursor was not, or not wholly, blood and tissue glucose, or else that the glycogen was formed from cleavage products which had been stored, not in the blood and tissue fluids, but in the liver itself, or in some other tissue. The possibility that the precursor of glycogen was present in the blood in the form of a hexose phosphoric ester, or as glucose *plus* a phosphate, was investigated by De Graff, Evans and Vacek [1932] and rejected. We have, rather as a forlorn hope, tried the infusion of an amino-acid and of products of starch breakdown, with saline infusions as controls.

(1) *Infusion of saline solutions.*

Six experiments were carried out in order to provide controls for the other experiments on infusion. In these the initial (calculated) glycogen concentrations ranged from 1·61 to 3·54 p.c. (mean 2·52 p.c.), so that the results, expressed in percentage change, should be comparable with those of the average curve for a large number of fasted cats. After decapitation, 25 c.c. of warm 0·9 p.c. NaCl solution in tap water was run into the femoral vein, from 10 to 20 min. being taken for the infusion. The results were not very uniform; in three cases the recovery rate was quite definitely greater than the average, in two it was about the same for 140 min. and then fell off, and in one it was poor throughout. The mean curve for the six experiments is given below (Fig. 8). It shows that, even when the average is taken, there is a definitely greater rate of recovery than in controls with no infusion and with about the same average initial glycogen content. The blood sugar also fell a little more quickly, but there was no very evident diuresis.

If these results can be interpreted to mean that the infusion has brought about an acceleration of glycogen recovery, as we think they can, it is probably better for the present to accept the fact and to let the explanation wait. Several possible ones suggest themselves, but have not been examined.

(2) *Infusion of alanine.*

The formation of glycogen from protein has long been accepted, and it has recently been shown by Wilson and Lewis [1930] that *d* or *d-l*-alanine, when given by the mouth to fasting rats, led to an increase of liver glycogen in 3 hours. It seemed worth while exploring the possibility

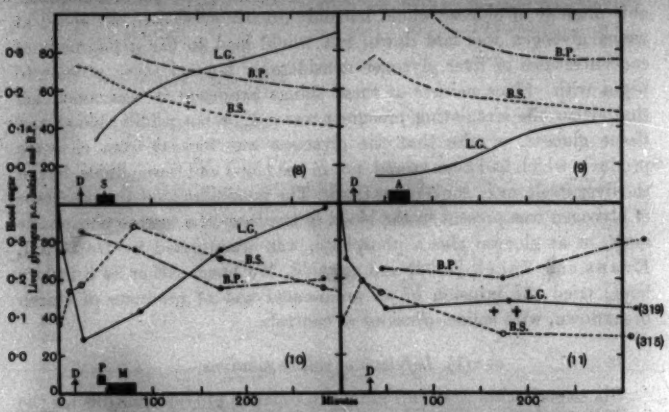

Fig. 8. Mean curve for six experiments in which 25 c.c. of saline solution were infused at *S*. *L.G.* liver glycogen; *B.S.* blood sugar; *B.P.* arterial pressure; *D*, decapitation.

Fig. 9. Mean curve for three experiments. Decapitate cats, infusion of alanine (2 g., 2 g., 4 g.) in 25 c.c. saline into femoral vein at *A*.

Fig. 10. Infusion of 2·5 g. of dextrin-maltose mixture at *M* into portal circulation of decapitate cat. Initial glycogen concentration = 1·68 p.c. *D*, decapitation; *P*, removal of spleen and insertion of cannula into splenic vein.

Fig. 11. 2·4 units insulin 21 min. before ether given. *D*, decapitation. Initial glycogen 5·23 p.c. Blood sugar before insulin = 0·083 p.c., 19 min. after = 0·085 p.c. The crosses show where series of convulsions occurred.

that, in our animals, glycogen might rapidly be producible from amino-acids, and, if this proved to be the case, it was conceivable that the glycogen formed in recovery might also arise from protein or from amino-acids.

Out of six experiments, a solution of *d-l*-alanine (B.D.H.) (2–4 g. in 25 c.c.) was infused, into the portal vein in one decapitate cat, and into the femoral vein in four decapitate and one decerebrate cat. The kidneys were first removed from one of the decapitate cats, and the urine of the three others was examined by formalin titration for alanine; in these

0·13 g. out of 2·5 g. injected, 0·38 g. out of 4 g. and 0·7 g. out of 3 g. were respectively found in the urine, and as these estimations included ammonia with the amino-acids, we may take it that less than 20 p.c. of the infused alanine was excreted. There was no evident dehydration of the tissues in these experiments.

The results of the experiments showed a slow loss of liver glycogen in the case of the decerebrate cat; in the five decapitate animals there was an increase which varied between wide limits. When all five experiments were averaged the curve was approximately the same as that given for untouched control experiments by Evans, Tsai and Young. But this result is largely due to the fact that one of the experiments gave an increase to 214 p.c. of the calculated initial value; this result should probably be rejected, for reasons already explained, on account of the low initial liver glycogen (0·42 p.c.). Another experiment (the portal vein infusion of 2 g. alanine) showed a slight loss of liver glycogen, but as this had an initial content of 7·85 p.c. glycogen, it might also be excluded. The remaining three, with initial glycogen from 2·5 to 3·5 p.c., which might be considered comparable with the saline injections, gave recoveries definitely below the average for non-injected animals and much lower than those injected with saline solutions, as is seen from Fig. 9. The blood sugar curve also fell rather less quickly than usual. The well-sustained arterial pressure shows that the loss of glycogen was not due to a poor circulation.

These experiments certainly do not lend any support to the idea that glycogen can rapidly be formed from alanine in the decapitate cat when it is injected into the general circulation, and we have not pursued them further.

(3) *Infusion of products of digestion of starch.*

In two experiments an attempt was made to find whether glycogen could be rapidly built from some of the intermediate carbohydrates, since it is possible that some of the products of glycogenolysis which may be present in the blood after decapitation might consist of larger molecules than glucose. The experiment had further interest in the possibility that such larger molecules might form part of the normal absorption products from the digestion of starch, suggestions of the presence of which in the portal blood have been made by several of the earlier observers (*e.g.* Pavy).

For the infusion, a mixture of maltose and dextrins was prepared by acting on soluble starch solution with saliva at body temperature until

the achromic point was reached. The solution was then boiled, filtered and diluted down to make a 10 p.c. solution relative to the initial starch. The results of one experiment, in which 25 c.c. of solution were slowly infused into the portal (splenic) vein, are given in Fig. 10.

The results are similar to those reported by Evans, Tsai and Young for the injections of glucose into the portal vein and do not differ significantly from those in which a simple saline solution was infused.

It seems doubtful, therefore, whether, in the short duration of these experiments, the natural rate of restitution can be accelerated by either glucose or malto-dextrins. In the later stages, not shown in the graph, the glycogen content of the liver, as usual, declined again.

(4) *Previous administration of insulin.*

In view of the possibility that insulin may, under certain conditions, retard glycogenolysis in the liver, it was thought that there might be an increased amount of insulin present after decapitation, and that this might favour the deposition of liver glycogen, and, though both parts of this supposition seemed unlikely, we put it to the test. In a preliminary experiment we found that the subcutaneous administration of 2·4 units of insulin to an unanæsthetized fasted cat produced a slow lowering of blood sugar, from 0·076 p.c. initial to 0·063 p.c. in 1 hour and to 0·04 p.c. in 5 hours, and we accordingly chose this dose as being not too great for an acute experiment under our conditions. We did three such experiments, giving the insulin 20 min. before the etherization for the subsequent procedure.

The results were as we anticipated (Fig. 11), viz. a smaller rise of blood sugar on decapitation, a subsequent fall to a low level, and a retarded and small glycogen recovery (except in one case where the initial glycogen was very low). The loss on decapitation in both of the satisfactory experiments was not very great, which favours the suggestion that the insulin had checked the glycogenolytic process.

Though we might have tried the effect of smaller amounts of insulin, we did not think this worth while at the present juncture.

(5) *Replacement of the circulating blood by defibrinated blood.*

These experiments were undertaken because of their relation to perfusion experiments, for which defibrinated blood is so generally used. Evans, Tsai and Young [1931 *b*] found that glycogen recovery was favoured by infusions of defibrinated blood when this resulted in an improvement of a poor circulation. We have carried this further by

giving defibrinated blood, not only to supplement that already in circulation (four experiments), but also (in two experiments) to replace it as completely as possible, by several successive infusions and bleedings. The defibrinated blood was obtained from a second cat which was bled out in two or three stages for the purpose, the liver glycogen being followed to ascertain the effect of severe hæmorrhage upon it. The defibrinated blood when freshly drawn usually had a high sugar content, but this was not invariably so by the time the infusion was made, because of loss in the meantime by glycolysis.

It is not necessary to give details of the experiments in which defibrinated blood was added to that already circulating. It will suffice to say that they all showed recovery, though this was no greater than was to be expected without such additions. One of the effects of the infusion was a great enlargement of the spleen, and it would probably have been better to have removed this organ beforehand in such experiments.

The following examples will suffice to show that even after the substitution of the larger part of the animal's own blood by defibrinated blood, good recovery can still occur. This is rather remarkable in view of the severity of such treatment. The protocol for a donor animal (from a different experiment) also illustrates the rapid loss of glycogen during bleeding.

The effect of hæmorrhage on the glycogen content of the donor is of interest since it explains why such low initial glycogens have been obtained by those who have attempted to prepare the liver for perfusion, or for analysis, by methods involving considerable loss of blood. There can be no doubt that the loss is principally due to attendant liberation of adrenaline and to nervous influences from the anæmic centres acting directly upon the liver.

Exp. 205. *The donor.* Cat. 2·15 kg. Fasted 44 hours.

Min.		Blood sugar p.c.	Liver glycogen p.c.	Liver glycogen p.c. of initial
−1	Blood by ear puncture	0·115	3·2 (calc.)	100
0	Ether given	—	—	—
6		←	2·31	72
16		0·336	—	—
17–19	52 c.c. bled from carotid	—	—	—
20		—	0·61	19
28–31	Further bleeding, 10 c.c.	—	—	—
32		—	0·23	7
34		—	0·16	5
36	Killed			
99	Analysis of defibrinated blood (just before infusion)	0·329		

Exp. 212. *The recipient.* Cat. 2·45 kg. Fasted 44 hours.

Min.		Arterial B.P. mm. Hg	Blood sugar mg./ 100 g.	Liver glycogen p.c.	Liver glycogen p.c. of initial
−1	Blood by ear puncture	—	92	1·43 (calc.)	100
0	Ether given	—	—	—	—
9		—	—	0·93	65
14		—	253	—	—
25	Decapitation completed	—	—	—	—
31		135	275	—	—
35		—	—	0·45	31
48	Infusion of blood (0·351 p.c. glucose) into femoral vein begun	—	—	—	—
54	23 c.c. now run in, continued	160	—	—	—
62	Bleeding begun, infusion continued	—	—	—	—
71	Bleeding stopped (45 c.c. in all)	—	—	—	—
72	Infusion stopped (65 c.c. given)	75	—	—	—
74	Infusion of cats own defibrinated blood (0·324 p.c. glucose)	—	—	—	—
88	Infusion stopped (57 c.c. given)	145	—	—	—
93	Bleeding begun	—	—	—	—
95	Bleeding stopped (40 c.c.)	135	—	—	—
98	Infusion begun	—	—	—	—
103	Infusion stopped (32 c.c.)	—	—	—	—
113		—	—	0·90	63
116		115	299	—	—
238		65	115	—	—
243		—	—	3·14	219
331		—	—	2·34	166
336		60	101	—	—
400		52	080	—	—
405		—	—	2·67	186
437		40	089	—	—
439		—	—	0·57	40
441	Killed. Liver remaining = 54 g.				

In the infused animal the circulating blood was almost wholly replaced by defibrinated blood, as was shown by its incoagulability when drawn; but towards the close of the experiment slight coagulability had returned to it. In the other experiment there was less recovery than in this case, apparently because the initial glycogen was higher (5·75 p.c.), but all these experiments may be taken together as showing that the defibrinated blood exerts no peculiarly deleterious action on the glycogenic power of the liver. The chief drawback to defibrinated blood is the vascular shock which it produces. This is shown in Exp. 212, given in full above, by the fact that although the blood-pressure at 95 min. was 135 mm. Hg, and as a further 32 c.c. were subsequently infused giving the

animal finally 69 c.c. more blood than at the start, the pressure by 116 min. had fallen to 115 mm. Hg, and in subsequent periods was not well maintained. It was for this reason that we proceeded next to try the effect of various anti-coagulant substances on the glycogen restoration.

(6) *Addition of heparine.*

Infusions of heparine [Messrs Hynson, Westcott and Dunning], dissolved in 25 c.c. normal saline solution, were given into the femoral vein in three experiments. The doses used were 7·4, 14·8 and 29·4 mg. per kg. body weight. The blood in the first experiment clotted *in vitro* in 10 min., that in the other two remained incoagulable for 24 hours.

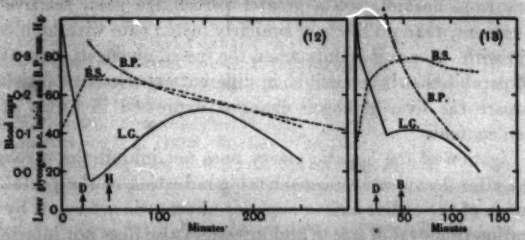

Fig. 12. Mean curve of three experiments in which heparine was infused.

Fig. 13. Mean curve of two experiments in which 6 c.c. of 8 p.c. Chicago blue were injected intravenously at B. Decapitation at D.

Recovery of glycogen was below the normal for the two higher doses and above for the smallest dose, but the initial liver glycogen was low in that case, so that supernormal recovery was to be expected. When averaged out the three curves gave recoveries below the normal average (Fig. 12). It would seem that the use of heparine is detrimental to the liver.

(7) *Addition of Chicago blue.*

Two cats, subsequent to decapitation, were infused with 6 c.c. of 8 p.c. Chicago blue [Rous, Gilding and Smith, 1930], previously boiled and filtered, and washed into the femoral vein with a small quantity of saline. There was loss instead of recovery of liver glycogen, the hyperglycæmia showed little reduction, the blood-pressure fell rapidly and the animal soon died (Fig. 13). The sample used appeared to be toxic to the liver, and further investigation was abandoned owing to the drawback this anti-coagulant has of preventing visual assessment of the degree of arterialization of the circulating blood.

SUMMARY.

1. Further consideration is given to the details of the methods used for the taking of liver samples for glycogen estimation. The changes observed after decapitation are not attributable to changes in the water or fat content of the liver. In experiments involving the taking of several successive samples of liver it is important, where changes of the total glycogen content of the liver are required ("balance-sheets"), to take into consideration the effect of the diminishing amount of liver tissue present, which makes glycogen gains smaller than they appear to be.

2. Livers of fasted cats with low initial glycogen content show on recovery from decapitation a greater percentage gain, relative to the initial content, than do livers of similarly fasted cats with high contents to begin with. Hence it is only when the initial glycogen concentrations are comparable that it is possible, in different experiments, satisfactorily to compare the liver glycogen changes, expressed in terms of initial glycogen content.

3. Ligation of the hepatic artery does not inhibit the glycogen deposition after decapitation; considerable reduction of the portal inflow, by ligation of the cœliac axis or superior mesenteric artery, or by partial evisceration (removal of spleen and intestine) also does not interfere with the recovery, but simultaneous ligation of cœliac axis and superior mesenteric artery does stop recovery. Occlusion of bile exit along the bile ducts also does not inhibit recovery.

4. Infusion of 25 c.c. of normal saline solution into the femoral vein somewhat enhanced recovery, but infusion of 2–4 g. of alanine delayed it. Recovery of glycogen was not greatly, probably not significantly, increased by injection of 2·5 g. of a malto-dextrin mixture into the portal vein. Subcutaneous injection of 2·4 units of insulin 20 min. before the experiment was begun retarded recovery.

5. Replacement of the circulating blood by defibrinated cat's blood did not inhibit the recovery significantly, but injection of heparine or Chicago blue in anti-coagulant doses did inhibit it, the former slightly, the latter, with the sample at our disposal, completely.

We wish to thank Dr H. P. Gilding for the sample of purified Chicago blue.

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THE EFFECT OF SOME ACCIDENTAL LESIONS ON THE SIZE OF THE SPLEEN.

By JOSEPH BARCROFT.

(*From the Physiological Laboratory, Cambridge.*)

Observations made by Barcroft and Florey [1929] showed that the operation for exteriorization of a portion of the small intestine caused a shrinkage of the spleen which persisted for days and perhaps weeks after the actual operation. Moreover, the shrinkage was accentuated by perforation of the gut leading to peritonitis.

Further observations were made in which the abdomen was opened [Barcroft, 1931], a loop of intestine was exposed for a few minutes under aseptic precautions and replaced in the abdominal cavity, which was then closed. The result was a contraction of the spleen lasting for some days.

These operations may be contrasted with such a lesion as a hernia, which is followed by no contraction of the spleen. Fig. 1 shows the alteration which took place in the volume of the organ, (1) when the operation of excision of the superior cervical ganglion was performed, (2) when a hernia developed just ventral to the spleen, so that the abdomen was closed only by the integument, and (3) when the operation was performed successfully to cure the hernia. In the cases (1 and 3) of operation there was a marked contraction of the spleen which lasted some days. In the case of the hernia, however, caused presumably by a stitch giving way in the region of the spleen, there was no contraction. We do not know the precise stimulus which was responsible for the contraction. In the present case it was presumably a chemical one, for the spleen had been denervated. But granting the chemical nature of the stimulus, there remains unsettled the question of whether the stimulant was a product of tissue breakdown, of bacterial action, or was adrenaline which might have been liberated as the result of some action of nervous origin on the supra-renal bodies.

Four experiments were performed [Barcroft, 1931] in which the superior cervical ganglion was excised. In two cases the spleen had been

denervated during exteriorization, and in two others it had not. In all cases the spleen contracted as the result of the operation on the neck. The contraction was more marked in the innervated spleen, but not significantly so. It was hoped that by observation of the pupil on the side from which the ganglion had been removed, some information would be obtained as to the presence in undue amount or absence of adrenaline

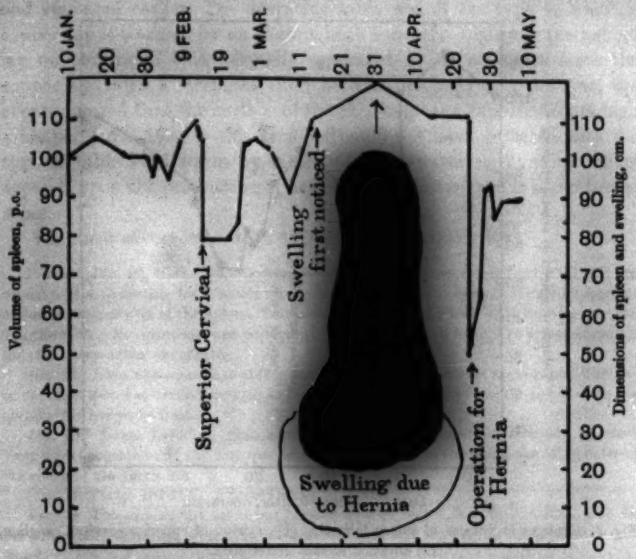

Fig. 1. Volume of spleen following (1) excision of superior cervical ganglion, (2) development of a hernia, (3) operation for hernia. Spleen reduced to seven-twelfths of its linear dimensions.

in the blood. This hope was not fulfilled, the dog being an unfavourable animal for observations on the sensitization of the pupil to adrenaline.

Another dog (Nina), the spleen of which was denervated during exteriorization, also yielded an interesting result. The superior cervical ganglion was removed and the spleen contracted and was regaining its normal size when, nineteen days after the operation, it again suddenly shrank; the bandage had caused a considerable abrasion under the neck.

It took nine days for the wound to heal, during which time the spleen remained shrunken and relatively colourless. Apart from the fact that the spleen had been denervated, the interesting points about the lesion were that it was purely superficial and not severe (Fig. 2).

During the winter of 1931–2 a phenomenon appeared in a number of animals which suffered from necrosis of considerable areas of skin. The necrosis developed only in those animals on which operations had recently

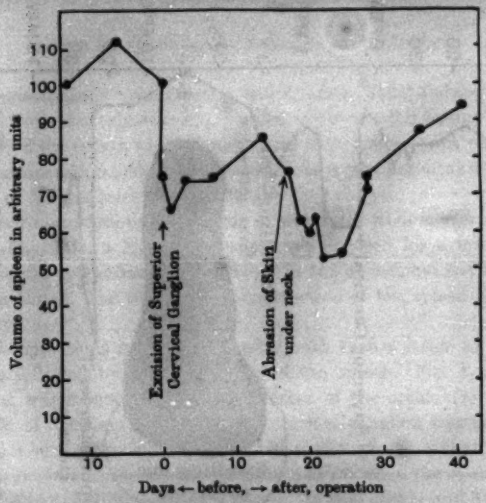

Fig. 2. Changes in volume of spleen following (1) excision of superior cervical ganglion, (2) abrasion of skin under neck.

been performed. Usually about a week, but in one case three weeks, elapsed before it became evident that a large patch of skin was dead and had to be removed. When that was done and the bare area dressed systematically the animals made a healthy recovery. The injury was quite superficial and the animals did not appear to be in pain, their spirits and appetites being excellent.

Without going into the various possibilities which might account for this phenomenon, the fact that it was confined to a certain category of animals ruled out a general epidemic. Experiments were instituted to test whether the operation had so lowered the resistance of the animals

that they fell a prey to infection which other dogs defied. The first of these experiments showed the true nature of the trouble. In order to obtain controls two animals were given an anæsthetic each for an hour and subjected to all the routine procedure of an operation, but no operation was actually performed upon them. These two, so far from experiencing no trouble, were much more seriously affected than the rest. The areas of necrosed skin were larger, the damage went deeper and appeared earlier. The operating table was of the type in which a copper top is warmed by an electric bulb beneath. Although the animal lay on perforated zinc normally separated by an air space from the copper and with a sterilized cloth between its body and the zinc, the events showed that the surface of the skin had been exposed to too high a temperature. Clearly this accident could not have occurred with the type of table kept warm by means of a hot-water tank, as the effect depends upon the production of heat at a rate faster than it is conducted away.

The most severe case, that of Nina, may be taken first.

Protocol. Jan. 20, 1932. Nina (spleen exteriorized and denervated Nov. 20, 1929) was placed on the dissecting table under C.E. mixture, as when animals are normally operated on for exteriorization of the spleen. The perforated tray and cloths were between her hair and the table. No operation was performed. She lay so for an hour. The spleen shrank in the usual way under anæsthetic.

Jan. 22. Nina was somewhat stiff in her gait and dull in general appearance. The hair on the right side was darkish looking as though a burn might develop, but as yet there was nothing further to be seen.

Jan. 23. Temp. 103·8° F. Skin nowhere broken, but two areas obviously necrosed. They were congested and the hair was loose. One area was in the flank, the other between the ribs and the thigh.

Jan. 24. Temp. 102·2° F. Affected areas as in Fig. 3.

Jan. 25. The care of the areas was handed over to Major Linton, R.A.M.C., who did the subsequent dressings, removing the necrosed skin and tending the affected areas with Eusol.

Jan. 26, 27 and 28. The bare areas extended and it became evident on the 28th that the damage had penetrated to the muscle. Temperature rose from 102° to 103·6° F.

Jan. 29. Temp. 88·5° F. No appearance of pain. Vomited whilst being dressed; no appetite.

Jan. 29–30. Dog died.

It is worth putting on record, though of no great interest in the present connection, that the mental condition of the animal, though lacking its usual brightness, was quite tolerable, and apparently equal to that of many rather phlegmatic dogs. On both January 28 and 29 she took interest in what was going on about her, her rectal temperature on the 28th being 103·6° and on the 29th 88·5° F. On the latter day her

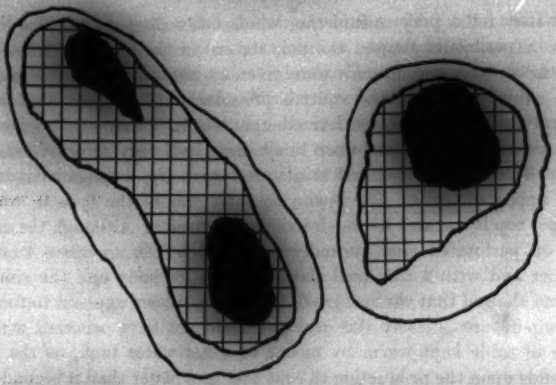

Fig. 3. Affected areas of skin in Nina. Black = skin dark. Cross-ruled = hair falling out (each square = 1 sq. cm.). Outer part = skin probably affected.

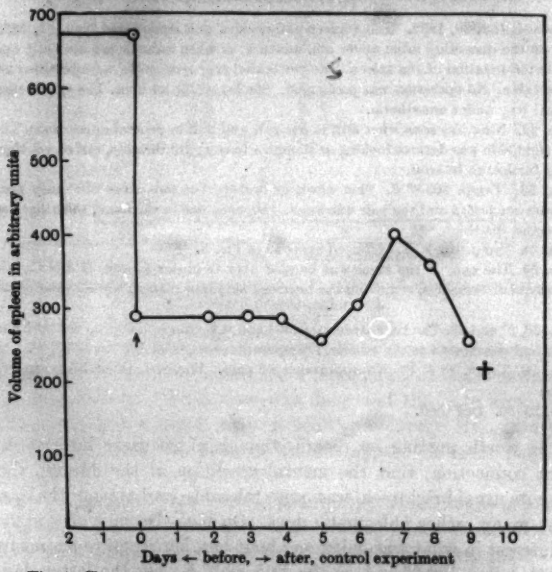

Fig. 4. Changes in volume of spleen of Nina after control experiment.

condition appeared bright enough to make me doubt the thermometer reading, and indeed not until the temperature had been taken three times and by two different thermometers did I accept it. Thus over a range of 15° F., or more than 8° C., the animal was quite *compos mentis*. The changes in volume of the spleen are shown in Fig. 4.

A similar experiment was performed on Bell on Jan. 21st, *i.e.* before the result of that on Nina had revealed itself. Bell differed from Nina

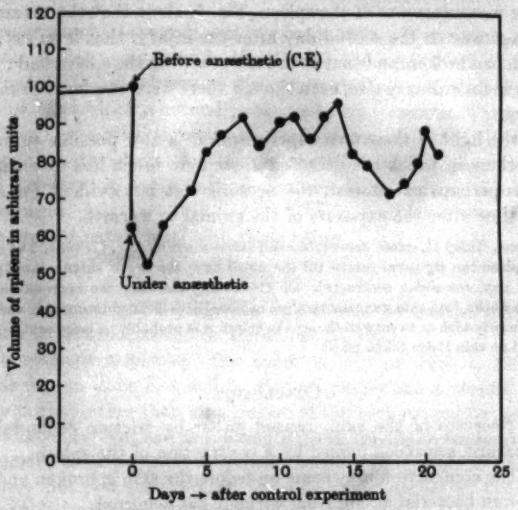

Fig. 5. Changes in volume of spleen of Bell after control experiment.

in three respects: (1) the spleen was not denervated, (2) the animal recovered, (3) the spleen only remained in a state of extreme contraction for a few days.

The lesions were of the same general nature as those of Nina, *i.e.* one in the flank and another behind the ribs.

The first portions of dead skin were removed on January 26th and the wound kept clean with Eusol. The animal "ran" rather a high temperature.

Jan. 27	Jan. 28	Jan. 29	Jan. 31
105° F.	104·2° F.	105° F.	103° F.

Protocol. Procedure as with Nina and earlier observations similar.

Jan. 31, 1932. Both areas were reported clean and the surfaces healthy and granulating.

Feb. 15. Area (1) flank dressed with amyl salicylate, area (2) with vaseline. This was continued up to Feb. 22, when the amyl salicylate dressing was stopped and both wounds were dressed with vaseline. In judging of the amyl salicylate dressing it should be said that while no benefit was derived from it, two facts must be borne in mind, firstly it was not used from the start and secondly it seemed less soothing—the dog was inclined to rub the area dressed with salicylate and not that dressed with vaseline.

Feb. 29. Area (1) healed, (2) healing rapidly.

Mar. 3. Both areas healed.

The measurements of the spleen, Fig. 5, show that the maximum of shrinkage was on the second day after the injury, that is to say, before the skin had broken, and that at the end of a week the spleen had returned almost to its ordinary size, even though there were two large granulating surfaces.

In the light of these two experiments it is now possible to interpret some others in which the necrosed areas were much less extensive, and what is perhaps of interest, the necrosis was not evident for a much longer time after the exposure of the animal to warmth.

Protocol. Tilley II, spleen denervated and exteriorized on Dec. 17, 1931. The shrinkage of the spleen ran its usual course till the ninth day; the organ shrank rapidly till the fifteenth day, remaining contracted. On the twenty-first day the necrosis made itself evident and the dead skin was removed, the necrosed area cleaned thoroughly and dressed. By the twenty-fifth or twenty-sixth day the spleen was probably as large as it would have been had no skin lesion taken place.

Conclusions.

1. Necroses of the skin, caused either by friction or unduly high temperature, are accompanied by a contraction of the spleen.

2. The contraction may come on before the skin is broken and therefore before bacterial action from without has occurred.

3. The contraction may pass off before the necrosed area has "dried up" if it is suitably dressed.

4. The above effects may be observed in denervated as well as innervated spleens.

The thanks of the author are due to the Royal Society for a grant from which the expenses of the above research have been paid.

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ALTERATIONS IN THE SIZE OF THE DENERVATED SPLEEN RELATED TO PREGNANCY.

By JOSEPH BARCROFT.

(*From the Physiological Laboratory, Cambridge.*)

In a former paper [Barcroft and Stevens, 1928 *a*, *b*] it was shown that the spleen in dogs contracts during "heat" and again towards the end of pregnancy, the usual size of this organ at these periods being little larger than that which is normally observed during exercise. The question of whether this contraction was nervous or humoral, or both, was not discussed. Another point which was unsettled in that paper was the rate of recovery; in some cases it was almost immediate, in others it was less rapid.

The present paper deals with these points.

Operative.

Bitch Nina, weight 6·8 kg. The spleen was denervated during the operation for exteriorization on December 20, 1929.

First observed pregnancy. She came on heat on June 1, 1930, and took the dog on June 3, 4 and 5. Her pregnancy ran a normal course, with no mishap other than an abrasion of the neck caused by her collar on September 1. She had five living pups in the night September 3–4. Nina suckled these puppies; they died, however, one by one. On the 20th there were but two, on the 22nd one which she suckled till October 10 (Fig. 1 A).

Second observed pregnancy. The spleen remained fairly constant in size from October, 1930 to January, 1931, if anything there was a slight increase in size: on February 2 Nina showed signs of coming on heat. The effect on the spleen was rather ambiguous, *i.e.* there was a trifling contraction of the organ, but it was within the range of ordinary casual variations. In fact no change that could be connected with reproduction took place till the actual commencement of labour on April 23. On the morning of April 23 the area of the spleen was 29·5 sq. cm.; after labour commenced it was 26 sq. cm. and about ten minutes after the birth of the first pup it was 24 sq. cm. Six pups were born weighing in aggregate 1·3 kg. The area of the spleen was 23 sq. cm. next morning and it reached

its minimum of 21 sq. cm. on April 27. It varied between that and about 25 sq. cm. till May 16, when it tended gradually to return to its original size. On June 6 the pups were removed. For some days, however, they had supplemented such diet as they obtained from the breast by partaking of the food given for the mother (Fig. 1 B).

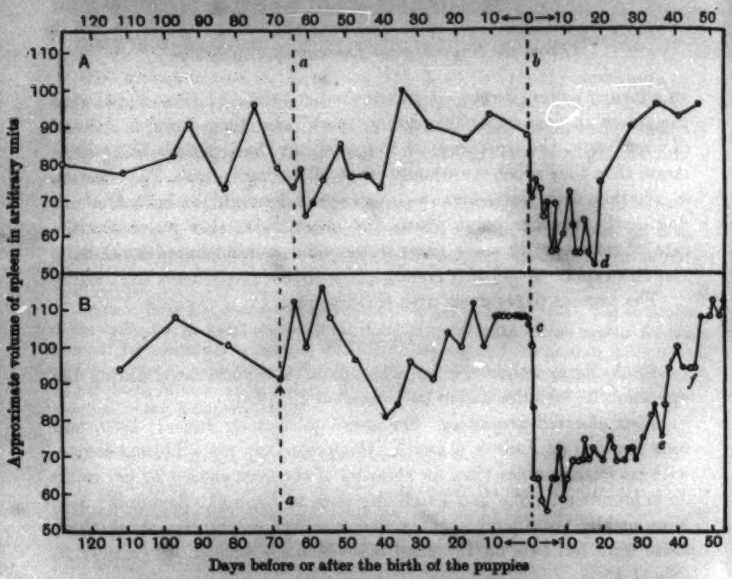

Fig. 1. Spleen denervated. A, first pregnancy; B, second pregnancy; (*a*) commencement of "heat," (*b*) five puppies born alive only one of which survived beyond the 18th day (*d*), (*c*) six puppies born alive of which four survived and were suckled entirely by the mother till the 36th day (*e*), and partially till 46th day (*f*).

Comparison of results obtained with innervated spleen.

The characteristic alterations in the size of the spleen, shown in Fig. 1, differ materially from those obtained in experiments on animals whose spleens had not been denervated. The outstanding facts are:

(1) In the denervated spleen there is no contraction during the later part of the pregnancy, whereas in the normal spleen the contraction in the last weeks is almost maximal.

(2) In the denervated spleen the alterations in size during "heat" are uncertain, whereas in the normal spleen there is well marked contraction.

(3) After parturition the spleen is contracted for a greater or less period whether it be denervated or not.

The present experiments seem to shed some light on the third of the observed phenomena. The contraction is evidently of uncertain duration, but it coincided pretty closely with the period of lactation. Further

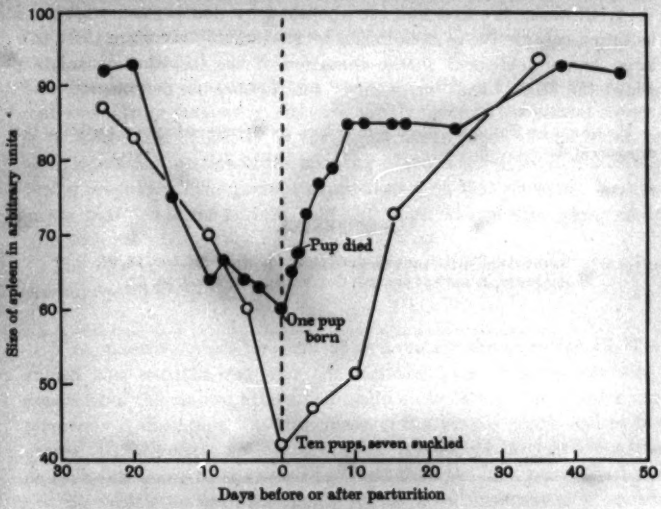

Fig. 2. Spleen innervated, correspondence between contraction of spleen and suckling of young.

evidence is to be found in support of this view from a consideration of some older experiments which were not understood at the time.

Fig. 2 shows the course of two pregnancies from about the sixth week onwards. In one the post-partural contraction was of short duration, in the other it was of long duration. In the first the bitch (Daisy) gave birth to but one pup which died on the third day, so that the demand for milk was practically non-existent; the other (Rastus) gave birth in the night to ten pups of which seven were found to be alive the next morning. She suckled the pups, but unfortunately no record was kept of the length of time.

SUMMARY AND CONCLUSIONS.

1. The contractions of the spleen which are associated with pregnancy in the dog can be divided into:

(*a*) The "heat" contraction.
(*b*) The pregnancy contraction.
(*c*) The lactation contraction.

2. Of these the first two are abolished by denervation whilst the lactation contraction is undiminished. Presumably therefore there is a large humoral element in the causation of the lactation contraction whilst the "heat" and "pregnancy" contractions are nervous.

My thanks are due to the Royal Society for a grant which defrayed a portion of the expenses of this research.

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THE VOLUME OF BLOOD IN THE UTERUS DURING PREGNANCY.

By JOSEPH BARCROFT and PAUL ROTHSCHILD.

(*From the Physiological Laboratory, Cambridge.*)

The discovery of Barcroft and Stevens [1928 *a*, *b*] that the spleen contracts during pregnancy, either to supply blood to the genital organs, or to contribute indirectly to the formation of blood in the fœtus or both, raises the question of the quantity of blood contained in the uterus during pregnancy. The present paper deals with that problem. Experiments were made on rabbits, cats and dogs. The present paper deals only with rabbits.

The experiments fall into two series according to the operative procedure employed.

Series 1.

The operative procedure was approximately the same for all. The animal was anæsthetized with C.E. mixture, 1 c.c. of blood was withdrawn from the carotid artery and made up to 50 c.c. The bladder was removed. A dissection was then made of the vessels which lead to the uterus. Usually there are five groups of vessels, one in the vagina, one on each side going to the broad ligament from the iliac vessels, and the ovarian vessels on each side going to the broad ligament from above. The general arrangement is given in Fig. 1[1].

A ligature was put round each of these groups of vessels loosely, with a "half-hitch." Great care was exercised lest the ligature should press upon the veins and cause congestion. At a given signal all the ligatures were tied simultaneously. If the operation was carried out successfully, all the avenues by which blood could enter or leave the uterus, vagina, Fallopian tubes and ovaries were closed simultaneously. A "Spencer-Wells" forceps was put on each group between the ligature and the animal, and the vessels were severed between ligature and forceps. The genital organs were then removed. No bleeding either from

[1] Fig. 1 is purely diagrammatic, but in one respect it may be wrong. Since I commenced to observe this point I have never seen the same number of embryos on the two sides.

the uterus or the animal should be caused by the removal. In case of bleeding from either the experiment is vitiated or, at least, there is a presumption of its being so. Should bleeding from the uterus occur, this would indicate an untied vein from which blood had already returned to the general circulation. In the case of bleeding from the animal there is the probability that an artery had been missed in tying the ligatures and that the uterus was therefore unduly congested. In the results

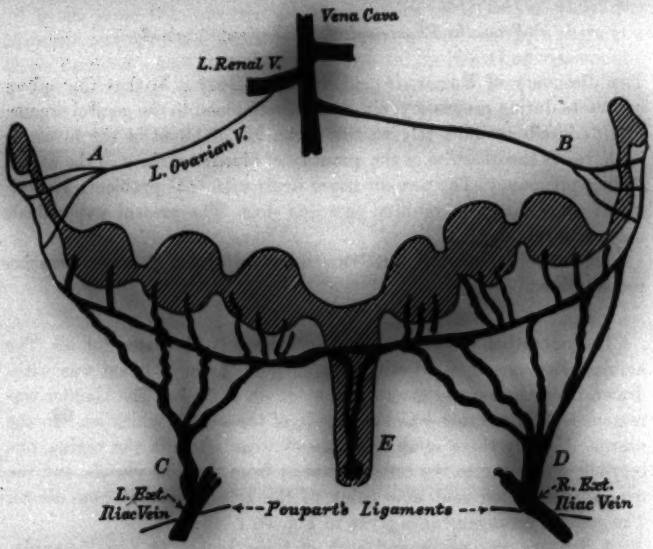

Fig. 1. Schematic representation of veins from female generative tract in pregnant rabbit.

appended no experiment has been included in which the genital organs did not come out cleanly. Four experiments have been omitted, on the ground that, since bleeding from the animal occurred when the vessels were cut, they all gave unduly high results.

The uterine vessels were opened up and washed out, then the uterus was cut up and thoroughly extracted with heparine saline. The extract, strained through muslin, was made up to a known volume and the blood present was estimated colorimetrically by comparison with the sample of blood taken at the beginning of the operation.

The results obtained from this series are tabulated in Table I and must be regarded as of a purely preliminary character, and indeed they might have been omitted entirely but for the facts (1) that they form an interesting basis of comparison with those obtained by the same technique on dogs and cats which will form the subject of a later paper, and (2) that certain individual experiments quoted in Table I will be referred to in the present paper.

TABLE I. Rabbits.

No. of exp.	Weight of animal kg.	Days since impregnation	Weight of uterus g.	No. of fœtuses	Wt. of fœtuses or young g.	Wt. of fœtal placenta g.	Blood in uterine vessels c.c.	Remarks
1	3·6	–	–	–	–	–	1·7	Not pregnant
2	2·7	–	7	–	–	–	0·6	,,
3	3·4	–	10	–	–	–	1·6	,,
4	3·1	–	4	–	–	–	0·6	,,
5	2·3	10	15	5	5	(–)	1·65	
6	2·2	11	12·5	8	7·5	(–)	4·00	
7	2·5		36	6	335	–	5·2	Clots in uterus
8	3·9	12	25	6	11	(–)	9·0	
9	2·75	22	92	11	106	(–)	11·2	
10	2·5	27	68	6	121	–	10·0	Oper. during birth
11	2·7	27	78	9	289	(–)	13·8	Clots in veins
12	3·5	28	75	8	321	(–)	20·0	
13	3·1	26	92	9*	360	(–)	26·8	
14	2·5	30	76	10	335	–	Bleeding in the uterus	
15	3·7	30	45	5*	170	–	21·0	
16	3·7	30	77	11	312	–	29·0	
17	3·4	27	69	10	283	48	21	
18	3·4	30	82	8	207	39	11	
19	3·0	28	95	8	232	41	13·4	
20	3·2	27	91	11	376	(–)	36	
21	3·1	16	36	9	8	(–)	2·7	
22	3·3	29	49	9	222	24	8·8	
23	3·05		–	6	340	–	6·2	1 day after birth
24	3·25	–	–	6	374	–	9·64	,,
25	2·20	–		6*	7	–	10·9	
26	2·25		–	7	135		14·1	
27		28?		8	48	–	12·5	
28	2·90	30	–	4	195	–	8·5	Just before birth

* Plus one rudimentary fœtus.

The principal finding was however of a somewhat negative character —only one correlation could be made out and that was of a very rough nature. It was a general relation between the quantity of blood in the uterine[1] vessels and the total weight of the fœtuses. This correlation is shown in Fig. 2 A which includes experiments in which the number of

[1] Unless otherwise stated the word uterine in this paper is used to include the whole female reproductive tract.

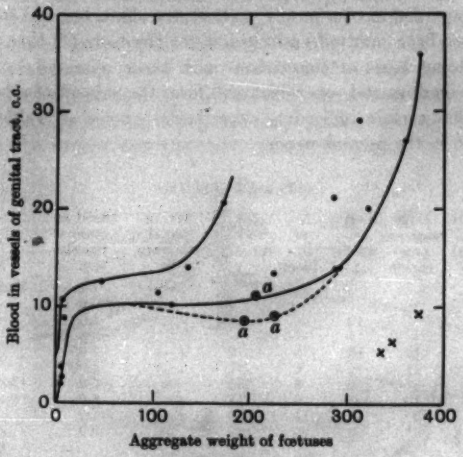

Fig. 2 A.

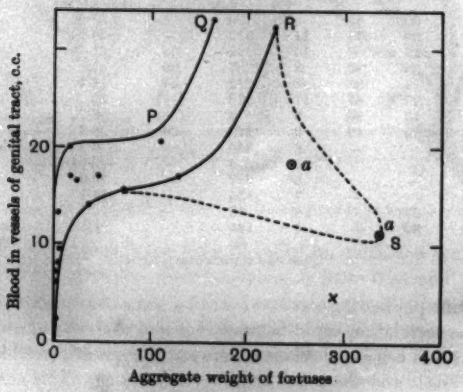

Fig. 2 B. For explanation of lettering see text.
Crosses = post-partem observations.

fœtuses have been from 6 to 11. When plotted a fairly definite sequence of relations comes out:

(1) The quantity of blood increases from about one to about 10 c.c. while as yet the weight of the fœtuses is insignificant.

(2) The weight of the fœtuses increases greatly in proportion to the quantity of blood.

(3) In the last phase the quantity of blood again rises relatively.

Towards the end of pregnancy there are 20–30 c.c. of blood in the uterus, but the day after parturition (the crosses in Fig. 2 A) the blood has very largely disappeared.

The bulk of the blood in the third stage is not actually in the uterus but in the great veins leading from it.

Series 2.

The experiments in series 2 were carried out by a technique evidently better than that employed in series 1, and which differed from the earlier methods in the following way:

Firstly, we were fortunate in being able to get in touch with a very reliable supply of rabbits, mostly Chinchillas. All the animals were

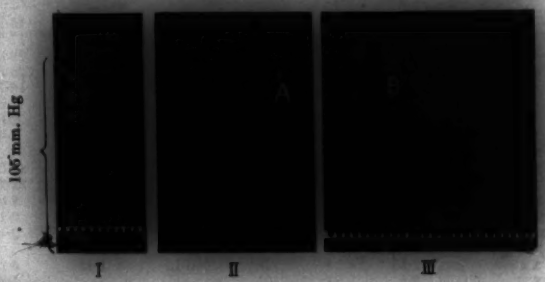

Fig. 3. General arterial pressure during certain periods of the dissection. Base line = bottom of black area. Time = seconds. I, at commencement. $p = 105$ mm. Hg. II A, insertion of ligature round vagina. III B, simultaneous tightening of all ligatures.

hardy, of very uniform weight and very carefully and reliably "stocked." A batch of six was stocked for us every week while we were at work, and very few rabbits failed to become pregnant.

Secondly, we were able to carry out the dissection and placing and tying of the ligatures without making more than a small local opening in the abdominal wall, so that in some cases the vessels had all been tied

up before we even saw whether or not the uterus was pregnant. Fig. 3 shows a tracing of the arterial pressure in such an experiment. The pressure was practically as high at the moment of tying the vessels as at the commencement of the experiment.

Thirdly, the experiments were carried out under dial, usually 2 c.c. injected into the abdominal cavity. Occasionally in addition a little C.E. mixture was given at the commencement of the experiment.

Fourthly, the hæmoglobin solution obtained from the uterine blood was filtered through Kieselguhr, a suggestion made to us by Dr Keilin. If about 10 c.c. of the filtrate are allowed to go to waste, the fluid which comes subsequently appears to have lost none of its hæmoglobin, whilst it is perfectly clear.

The data obtained in series 2 are given in Table II.

TABLE II.

No. of exp.	Wt. kg.	Days since impregnation	No. of fœtuses	Wt. of fœtuses or young g.	Wt. of fœtal placenta g.	Blood in uterine vessels c.c.
I	3·0	21	8	34·3	.	14·0
II	2·9	20	6	21·6	.	16·6
III	2·6	15	8	5·0	5·0	9·6
IV	Died 3·0	15	.	–	–	–
V	0·3	23	8	72·0	18·6	15·1
VI	2·8	22	7	42·0	14·0	17·2
VII	3·0	25	10	16·8	23·3	20·0
VIII	2·9	24	10	110·0	23·3	20·5
IX	2·7	26	5	127·5	16·5	16·7
X	2·9	25	0	–	–	2·6
XI	2·3	6	+	?	?	2·6
XII	2·4	6	+	?	?	3·0
XIII	3·7	15	11	3·3	7·2	?
XIV	3·1	29	8	236·0	20·5	32·3
XV	3·0	28	6	174·0	15·0	33·2
XVI	3·0	15	7	3·5	8·8	8·0
XVII	2·5	30	0	–	–	2·1
XVIII	3·1	29	0	–	–	2·1
XIX	Died on table	11	–			
XX	2·55	11	9	0·15	1·45	6·2
XXI	2·6	11	–	+	+	7·2
XXII		20	8	17·5	20·0	17·0
XXIII	2·9	30	9	337·0	22·5	11·4
XXIV	Died under dial	30	0			
XXV	2·4	30	6	290·0 (young)		
XXVI	3·3	30	0			
XXVII						
XXVIII	2·7	28	11	585·0 (young)	–	13·2
XXIX	3·0	30	7	245·0	21·0	18·4

This series was as fruitful in correlations as the previous one had been barren of them. The first to which we would draw attention is that

between the progress of the pregnancy in days and the quantity of blood in the uterus. This correlation is given in terms of the actual experiments in Fig. 4, whilst Fig. 5 gives the average obtained by taking the mean of the groups of black spots joined by dotted lines in Fig. 4.

The points to be noted are:

(1) That the maximum quantity of blood in the uterus is about 30 c.c. or perhaps $\frac{1}{3}$ of that in the rabbit.

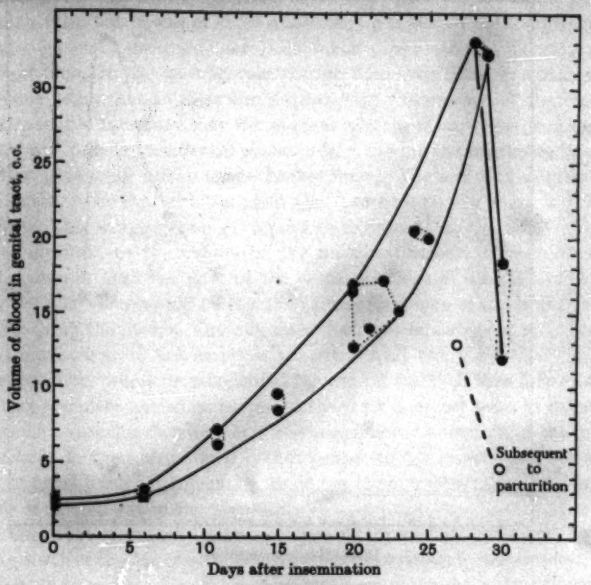

Fig. 4.

(2) That the vascularity of the reproductive tract commences to increase very early; certainly from the sixth day there is an appreciable rise in the volume of blood.

(3) That the peak is about the twenty-seventh to twenty-ninth day.

(4) That the volume of blood in the reproductive tract falls suddenly before parturition takes place. In this connection reference may be made to certain experiments in Table I. In experiment 10 labour commenced when the animal was on the table, though the record shows the

day as only the twenty-seventh. There were but 10 c.c. of blood in the uterus. Again in experiment 28 (Table I) the operation was on the thirtieth day and the contraction of the vagina suggested the imminence of labour; there were but 8·5 c.c. of blood in the reproductive tract.

The large drop in the quantity raises some interesting points in the consideration of which it is well to remember that we know nothing as

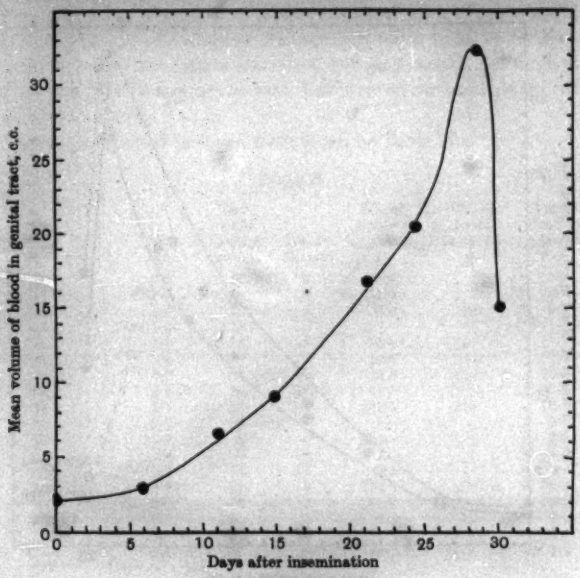

Fig. 5.

yet regarding the quantity of blood which circulates through the reproductive tract in a given time, as opposed to the quantity to be found in the uterine organs. It seems likely that the presence of 30 c.c. of blood is unnecessary either to the contractions of the uterus or to the actual life (over short intervals of time) of the fœtus. The question therefore may be asked what function so great a quantity of blood subserves in the uterine parts, especially as it is mainly to be found not in the organs themselves but in the large venous arcades and their

related veins. This question we cannot at present answer, but certain observations may be relevant.

Firstly, we have observed on more than one occasion that any undue pressure on the principal vein was associated with, and probably caused extensive intra-uterine hæmorrhages, on the same side as the venous obstruction. On one occasion there was some bleeding during the dissection from a little vessel in the region of the letter *C*, Fig. 1. The bleeding was stopped by pressure and the pressure involved the main uterine vein, but it was of short duration; on a second occasion when a similar accident occurred, care was taken never actually to stop the flow of blood in the uterine vein though doubtless there was sufficient pressure somewhat to raise the resistance to the passage of the blood. It is possible therefore that the copious system of anastomosing veins may be to prevent accidental pressure from raising the resistance locally and so producing intra-uterine hæmorrhages. The size and number of the embryos in the rabbit suggest that pressure on the veins is a real danger. This exaggeration of venous development in the mother is in great contrast to the calibre of the umbilical vessels. The naked-eye impression is that the sum of the sectional areas of all the umbilical arteries and veins would be less than that of any one of the larger veins which drain the uterus. One supposes that, at a late stage of pregnancy, the metabolism of the embryos constitutes all but a relatively small portion of the whole metabolism of the genital tract. If that inference is correct the mere exchange proper to the tract does not seem to demand so great a vascular development. The suggestion, however, that the main exchange in the uterine area is that proper to the growth and activity of the fœtal tissue is conjecture; we do not know whether work on a large scale is carried out in the placenta.

The great vascularity may be an index of some factor connected with the growth of the fœtus, as opposed to its mere existence. Other questions which at once arise are, What causes this pre-partural cutting down of the blood in the genital organs? Is the loss of blood a link in the chain which ultimately ends in labour? The answer to these questions we do not know.

Another correlation which we have observed is that between the progress of the pregnancy and the growth of the fœtus. This matter has already been the subject of observations as yet unpublished by Hammond [1931]. Hammond has very kindly shown us his figures of which ours afford a general confirmation (not that this is necessary); see Fig. 6.

A third correlation which we believe to be new is the relation in time between the vascularity of the uterus and the growth of the embryos. This is also shown in Fig. 6, depicting not only the weight of fœtal tissue expressed as a function of time, but also the parallel increment (over and above the normal 2 c.c.) of blood in the generative tract.

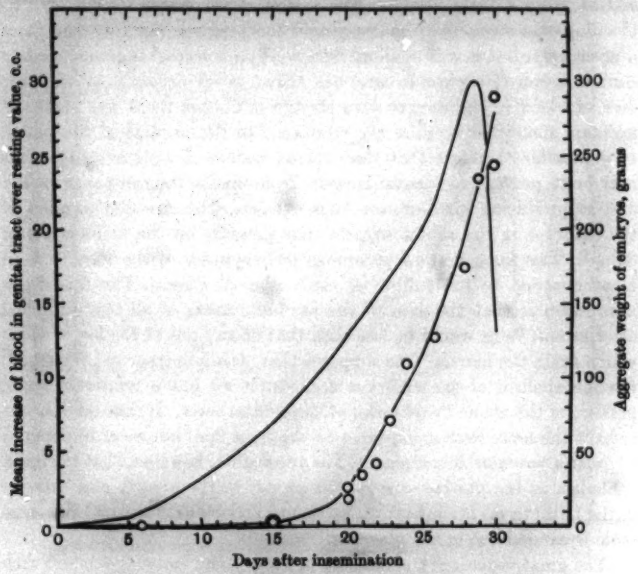

Fig. 6. Circles show the weights of the embryos. The line, without circles, the increment of blood.

The outstanding facts are that, whereas on the twentieth day the embryos are still of almost negligible bulk, the blood present has already attained to half its maximal volume, and indeed to a volume as great as on the thirtieth day.

An attempt to go further and correlate the blood volume in the maternal organs with either the number of embryos or the average weight of each embryo is less successful. On the whole on any particular day the more embryos there are the heavier their aggregate weight

appears to be (this is also a confirmation of Hammond's observations), but the proportion is not simple.

As a first approximation, though perhaps not in the final analysis, it seems fair to say that, on any particular day, the blood volume is almost independent of the number of embryos whilst the aggregate weight of the fœtuses is dependent on, but not a simple multiple of, the number.

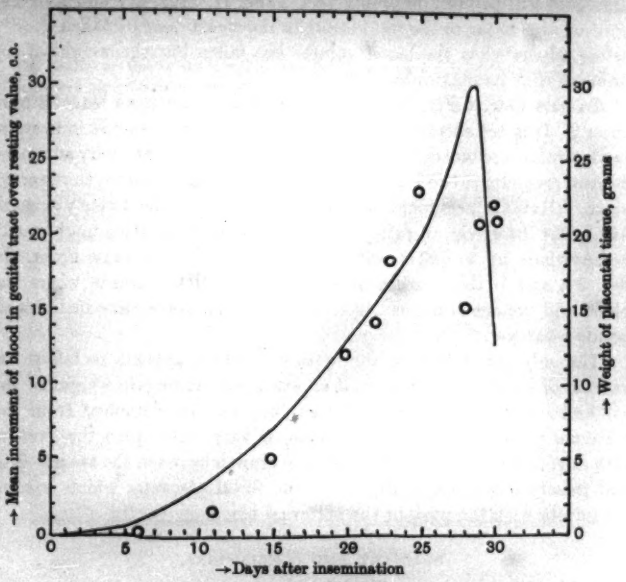

Fig. 7. Circles indicate the weight of the placentas. The line indicates the increment of blood.

We are now in a position to interpret Fig. 2. First let us consider Fig. 2B, in which the second series of experiments is plotted. We see that the first phase represents the first fortnight or so of the pregnancy in which the blood volume is increasing, while as yet the embryos are of negligible weight. The second phase represents the period from about the sixteenth to the twenty-fifth day, when the rate of fœtal growth is great proportionately to the increase in the blood. That this portion should be nearly horizontal is not surprising. More difficult matters are the

steepness of the final limb of the curve *PQRS*. Why in fact does the top of this portion stretch so far to the left as at *Q* and the bottom so far to the right as at *S*? Taking these points in order, the width of the distance *QR* is due to the fact just stated, that the maximal quantity of blood occurring about the twenty-eighth day will be about the same (30 c.c.) whether there be six embryos or eleven, but the weight of embryos in aggregate will be very different. (See Table II, exps. XIV and XV.) The position of *S* so far to the right is due to the measurements taken shortly before labour when the blood volume has fallen but the weight of the embryos is at its maximum.

To pass now to Fig. 2 A in the light of what we have learned from series 2. It is not surprising that the points marked *a* should indicate so small a volume of blood, they are from rabbits observed at a very advanced stage of pregnancy, two being on the thirtieth day and one on the twenty-ninth. (Rabbits are sometimes born as early as the twenty-seventh day.) The litters are all rather light, the points in question might easily be anywhere up to 400 g. with blood volumes of the same order. In Fig. 2 A and B the continuous lines enclose all the points where the blood and volume is increasing or maximal, the dotted line includes all points whatever.

The only correlation on the fœtal side which appears to follow the volume of blood in the maternal organs is the aggregate weight of the combined fœtal placentæ, so far as they can be detached from the maternal portions, and this correlation is very close up to the twenty-fifth day, but we have not obtained any growth between the twenty-fifth and twenty-eighth days (Fig. 7) in the fœtal placentæ which is commensurate with the peak in the maternal blood.

Summary and conclusions.

1. A technique is described for the measurement of the quantity of blood in the genital tract of the female rabbit.

2. During the resting condition the genital organs contain less than 2 c.c. of blood. They become appreciably more vascular from the fifth or sixth day onwards, and about half-way through pregnancy contain about 10 c.c. of blood. Up to this point the embryos are of negligible weight. By the twentieth day the embryos weigh only about 5 g. and the generative tract of the mother contains about 15 c.c. of blood.

The maximal quantity of blood, about 30 c.c., seems to occur about twenty-eight to twenty-nine days and the quantity falls rapidly before parturition. The significance of the arrangement of vessels is discussed,

especially in reference to the possibility of intra-uterine hæmorrhage due to pressure on the veins.

3. The quantity of blood in the generative organs on any particular day does not bear so near a relation to the number of fœtuses as does the total weight of the embryos.

4. The quantity of blood in the genital organs seems to be closely related to the combined weights of the fœtal placentas up to the twenty-fifth day when the latter seem to cease growing.

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OBSERVATIONS ON THE PROXIMAL PORTION OF THE EXTERIORIZED COLON.

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In previous papers it has been shown that the exteriorized spleen [Barcroft and Stephens, 1927] and loops of the small intestine [Barcroft and Robinson, 1929] of the dog yield valuable information concerning the function and activity of these organs under normal environmental conditions. Consequently it was of interest to study in a similar manner the activity of the exteriorized cæcum and proximal portion of the colon.

Although there may be some doubt as to whether the activity of the cæcum and colon under these conditions is exactly normal, we believe that our results are fully as valuable as those obtained by such methods as opening the abdominal cavity and surrounding the intestines with warm saline [Alverez and Zimmerman, 1927], or inserting balloons into the lumen of the intestine and recording movements on a smoked drum, as originally done by Bayliss and Starling [1901] and more recently by Templeton and Lawson [1931], or even the still more satisfactory method of Cannon [1902], who X-rayed the intestines after the administration of a barium meal. This last method, no doubt, has an advantage over the other two in that the animal can be observed under normal conditions, and that anæsthetics need not be given nor the animal sacrificed; but it too has its disadvantages in that the observations are indirect and made not on the gut itself but on its contents. Also the weight of the barium in the intestine may interfere with the normal movements.

Our method, we believe, though it has its own disadvantages, may add to the knowledge already obtained by the X-ray method in that (*a*) we can make observations on the intestinal muscles themselves rather than on their contents; (*b*) we study the animal, not under anæs-

thetic or during an operation, but after the animal has returned to normal health and activity; (*c*) we can study the same animal over a long period of time under various conditions, such as starvation, response to foods, and the effects of various drugs, such as morphine and magnesium sulphate, and their relation to the water content and movements of the colon.

Our data represent observations on three dogs. Before presenting the data, however, we consider it important to give an account of the operation and the time and progress of the dogs in their return to apparently normal activity. In each case a female dog was used, and no food was allowed for a day before the operation. One-half c.c. of 5 p.c. morphine was injected 1 hour before the operation. The anæsthetic used was an ether-chloroform mixture.

The steps of the operation were as follows: a transverse incision was made through the skin and abdominal wall about 1 inch above the umbilicus, extending about 2 inches on either side of the midline. At each end of this incision we cut away a circular piece of the abdominal muscle wall so as to avoid undue constriction of the ileum at one end and the more distal colon at the other. Then an opening of about the same size was made in the omentum and the colon located. The colon is usually found rather deep in the abdominal cavity and just below the lower curvature of the stomach, and may be recognized by the longitudinal striations of its muscular wall, which are more pronounced than those of the small intestine. After it was found and drawn to the outside, the cæcum was uncoiled slightly and anchored to the outside of the abdominal wall with one or two sutures. A number of catgut sutures sufficient to pull the abdominal wall together underneath the exteriorized colon were then put in place but not tied. Following this, other sutures were put through the skin in the same manner. Then, while the colon was held up in position, the muscle sutures were drawn up and tied, and then those of the skin. Care was always taken in placing the sutures to avoid constriction of blood vessels underneath the exteriorized portion, and at the same time, not to draw them too tightly. The total length of the exteriorized portion was about 12 cm., of which the cæcum made up 2½ cm.

After the operation was completed, the exposed portion was smeared liberally with vaseline, and supported on either side by a thick pad of cotton wool. These pads were held in place by a bandage wrapped tightly around the animal's abdomen. Clean bandages were put on every other day for at least the first 2 weeks, and the area washed with warm

saline, except when any sign of infection occurred, at which Eusol was substituted. The animals were kept in a warm room at a temperature of about 28° C. for at least a week after the operation, and to keep them quiet small doses of morphine were occasionally injected during the first 2 days. The rectal temperature was recorded daily. In regard to food, the animals were given water, usually containing glucose, on the second day; on the third day they were allowed both water and diluted milk, and on the fourth a small quantity of boiled fish. This fish diet was continued for nearly 2 weeks before more solid food was given.

The first fæces in each case were found in the cage on the morning of the fifth day, this being coincident with and probably related to an increasingly solid diet. As was expected, the fæces were of a very watery consistency, which continued from 10 days to 2 weeks and then gradually became more concentrated. To obtain information on the rate of concentration of the fæcal material from this time on, the water content was determined by collecting two samples of fæces immediately after defæcation, in weighing bottles, and drying them at 100° C. The rate of concentration of the fæces of the three dogs and the number of days required to reach a constant concentration are recorded in the following table.

TABLE I. The rate at which fæces concentrate after the operation.

Dog I—"Susie"		Dog II—"Florence"		Dog III—"Nina"	
Days after operation	H_2O of fæces, av. 2 samples (p.c.)	Days after operation	H_2O of fæces, av. 2 samples (p.c.)	Days after operation	H_2O of fæces, av. 2 samples (p.c.)
17	77·8	15	84·8	19	84·6
19	70·0	22	79·0	24	80·2
26	64·7	27	72·4	31	72·0
52	61·9	34	71·3	80	71·7

As Table I indicates, within 12 days after the fæces were noticed to be less watery the water content of the fæces had reached a level, this level being determined by comparing the last samples with some taken at a random time after apparent recovery of the dogs.

In conjunction with this watery state of the fæces, it was noticed that there was very little sign of activity in the muscular walls of the colon, and also that the area appeared to be congested and inflamed. It is possible that this condition was due to a loss of muscular tone brought on by the manipulation of the colon during the operation. But as the colon gradually took on a more healthy colour, a series of small kneading contractions were detected; at the same time the colon appeared to

become more distended, and soft and flabby to the touch. This state of recovery gradually spread back toward the cæcum, which regained its tone and normal colour about a week after the colon. The interesting fact in this connection is that at the time the colon and cæcum were regaining their tone and activity the fæces were becoming more concentrated, which is quite in keeping with the idea that the proximal colon and cæcum are closely related to the concentration of the fæces.

Another point of interest is the manner in which the act of defæcation was carried out, particularly by the dog "Susie," before and after the activity of the colon had been re-established. Previous to the recovery, it was noticed on many occasions that, whenever the dog was about to defæcate, she would run around the room in a distressed manner and at intervals slow down her pace and shake her hind quarters and tail most violently. This act she repeated four or five times, and then, without even squatting, would pass a thin watery mass of fæces with considerable force. But after the tone and activity of the colon and cæcum had been regained the act of defæcation was carried out quite normally. We also observed this same kind of behaviour on the part of the dog later when we were studying the activity of the colon after the administration of morphine.

These observations lend evidence to the view advanced by Hertz and his collaborators [1907] that, just previous to the act of defæcation, the proximal colon and cæcum contract, forcing the fæcal matter down into the rectum. Up to the present it has been impossible to observe the colon during actual defæcation, but we have observed it just before and after the act and found that the colon is very much distended before and emptied after. It has also been noted on several occasions that if the colon was kneaded for any length of time and the fæces thereby pushed along toward the rectum, upon releasing the dog, the act of defæcation took place.

Within 3 weeks after the operation it was noticed that an epithelial layer, starting from the cut edges of the skin, was growing over the exteriorized portion, and finally covered it completely. This is the same type of protective layer as covers the exteriorized spleen (Barcroft).

In discussing the types of movements found in the colon and cæcum, one is confronted with the difficulty of finding a satisfactory means of presenting the data. Moving pictures would be ideal for demonstration, but impractical for publication. Graphic records, although convenient, present mechanical difficulties in representing the true activity of the organ and are often misleading. We concluded, therefore, that direct

observation with the eye and a descriptive tabulation of the data were the most convenient and satisfactory for our purpose.

As any extraneous noises disturbed the dogs, we carried on our observations in a practically soundproof room. The animal was studied lying on her side on a table, the time of observation often lasting 1½ hours. We made observations of movements occurring (*a*) from ½ to 12 hours after feeding, and while feeding the dog on the table to note the presence of any gastro-colic reflex, (*b*) before and after giving her 2 g. of magnesium sulphate in 150 c.c. of water by way of stomach tube, and (*c*) under the influence of morphine. Since these three types of conditions produced different effects we shall treat them separately. The following discussion and figures will represent the findings on the two dogs "Susie" and "Florence." All the types of movements found were confirmed in the third dog, but not to such a pronounced degree, due to improper healing of the epithelium of the colon.

(*a*) *Normal movements of the cæcum and colon.*

The type of movements most frequently seen in the cæcum and colon occurred ½ to 12 hours after the animal had been fed. These movements started in the cæcum, where slight but definite kneading contractions occurred in the lower end, appearing three or four times, at intervals of about ½ minute. These were followed by a deeper contraction which involved practically the whole cæcum and usually had a forward movement, pushing on down toward the ileo-cæcal region. Then there was a tendency for the cæcum to relax, but shortly after, another deep cæcal contraction appeared, and often a third and fourth. They sometimes followed one another so closely that the cæcum appeared to relax hardly at all.

By this time there was usually a marked constriction in the region of the ileo-cæcal valve which remained contracted for 3 or 4 minutes. As a result of these cæcal contractions the colon became quite distended, and shortly afterwards one might see shallow anti-peristaltic waves appearing near the region where the colon entered the abdominal cavity. They continued to course back toward the ileo-cæcal constriction at a rate of five to seven a minute, becoming deeper and deeper as they continued along the colon. By this time the cæcum and ileo-cæcal constrictions were usually beginning to relax. After these anti-peristaltic waves had coursed back for about 2 to 4 minutes, a series of deep kneading contractions, about 1 cm. apart, appeared to start near the ileo-cæcal region, and work on down the colon, stopping the progress of the anti-peristaltic

waves. In some cases, by the time these kneading constrictions had reached the distal end of the colon, the ileo-cæcal region had become quite relaxed, while in other cases the whole colon seemed segmented at one time.

A second type of movement which we observed frequently had its origin in the ileo-cæcal region. It was a deep massive contraction that nearly occluded the lumen of the colon, and then very slowly passed on down the colon, forcing a mass of intestinal contents before it. As this

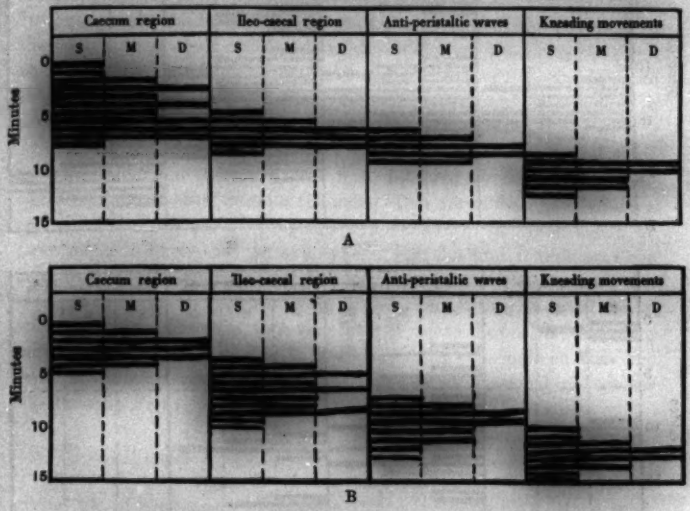

Fig. 1 A and B, The normal type of movements found in the cæcum and colon in dogs "Susie" and "Florence" respectively. S. Shallow contractions. M. Medium contractions. D. Deep contractions.

contraction moved along, there appeared in the distended portion of the colon just ahead of the contraction, about 1 cm. in length, a group of hardly perceptible anti-peristaltic waves. The total time it took the contraction to travel the length of the exposed colon was about 15 minutes. It seemed to occur when the fæcal content was rather dry and solid, for on no occasion was it observed when the colon was well distended with a watery fæcal material.

Quite different from these results were those obtained directly (2–5 min.) after the dog was fed with a biscuit or two on the table. In

this case the contractions first occurred in the ileo-cæcal region and colon, and not in the cæcum as before. These constrictions became very pronounced and were usually followed first by the anti-peristaltic waves and

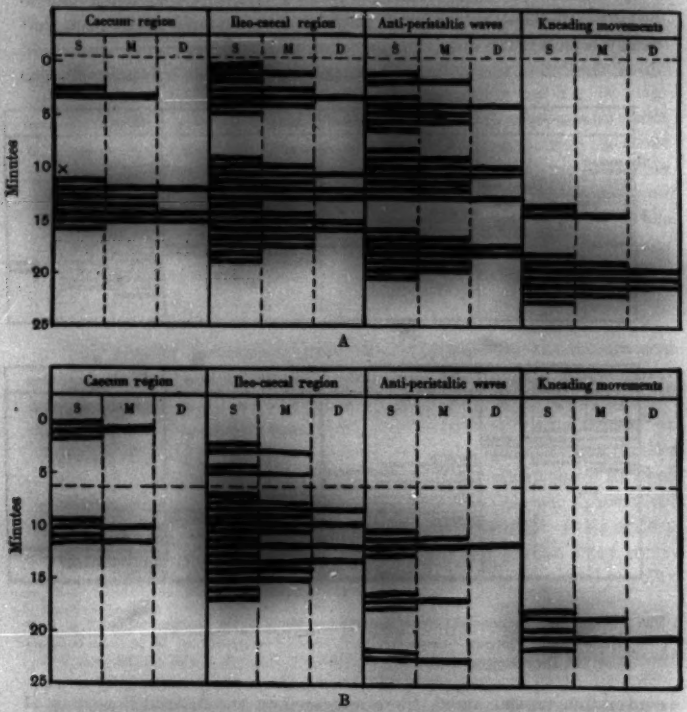

Fig. 2 A and B. Evidence of a gastro-colic reflex. The dotted horizontal line represents the point at which food was given under observation. (X) in Fig. 2 A is the point at which normal series of movements appears. (See Fig. 1.)

then by occasional kneading contractions. Then, with short periods of slight relaxation between constrictions, this series of movements was repeated again and again for about 10 minutes. The cæcum all this while remained comparatively inactive, in some cases for 30 minutes after the food had been given.

This type of contraction corresponds to those described by Hertz and Newton [1913], who, using X-rays on humans, found that similar movements occurred directly after the entrance of food into the empty stomach, and explained it as a gastro-colic reflex to clear the colon of material for the entrance of further intestinal content from the small intestine.

To present these two types of intestinal movements in a graphic form we have prepared the preceding figures (Fig. 1 A and B and Fig. 2 A and B). In these graphs the lengths of the horizontal bars represent the depth of the contractions, their positions on the vertical line indicating the times at which they appear. However, the number of bars does not indicate the number of contractions which occurred; the graph is designed to show the intensity of the movements and not the quantity.

Fig. 1 A and B give examples of the normal movements, and show how the cæcal movements appear first, followed by those in the ileo-cæcal region and then those in the colon. This graph does not include a representation of the slow progressive movement in the colon which we have referred to as the "second type." Fig. 2 A and B represent the gastro-colic reflex shortly after the food has entered the stomach, the major movements in this case being contractions of the ileo-cæcal region and anti-peristaltic waves, with very little evidence of cæcal and kneading movements. At (×), in Fig. 2, however, it may be noticed that the cæcal movements again appeared, starting a series of normal movements like those in Fig. 1.

(*b*) *Effects of* $MgSO_4$.

Observations were made on the colon and cæcum before and after giving 2 g. of magnesium sulphate in 150 c.c. of water by stomach tube. The results obtained can be best described by the use of Fig. 3 A and B.

As will be noticed, the usual cycle of movements appeared before the injection. As shown in Fig. 3 A, which represents the dog "Susie," within 15 minutes after the injection we found movements like those described in Figs. 1 and 2, the cæcum being very inactive and the other parts quite active. Three hours after the injection, however, the colon and cæcum were observed to be very much distended and less active, the only movements noticeable being shallow anti-peristaltic waves, appearing quite regularly and occasionally developing into deeper waves; and at times contractions of the ileo-cæcal region were also observed. The cæcum at this time was very much distended and practically inert,

and there were no kneading movements following the anti-peristaltic waves. About 5 hours after the injection, the movements were similar to those just discussed, except that there seemed to be more activity in

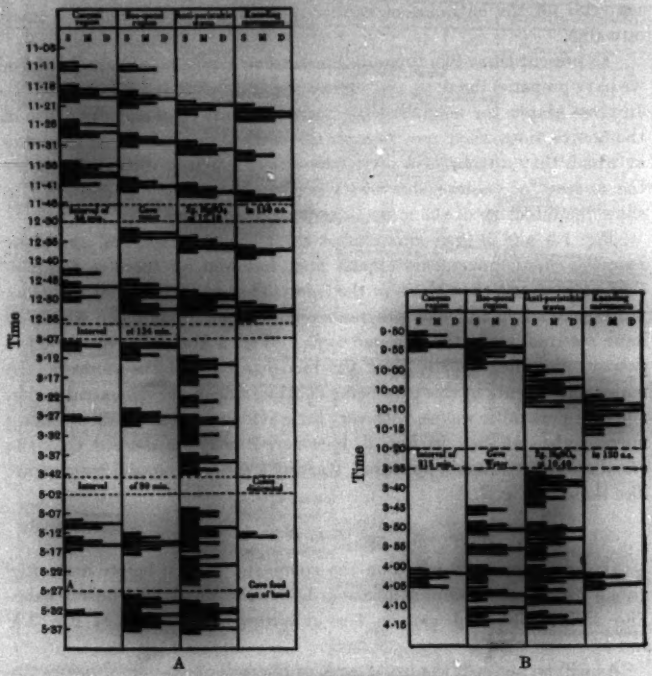

Fig. 3 A and B. The effects of magnesium sulphate by stomach tube with observations at different intervals of time after the administration.

the cæcum than before. With this dose of 2 g. of magnesium sulphate, defæcation took place from 5½ to 6 hours after its administration, the fæces being very watery, as would be expected. In one case the colon was observed directly after defæcation and was found to be empty and very inactive.

(c) Effects of morphine.

Since it is generally accepted that morphine has an inhibitory effect on the intestinal movements, and is often prescribed in cases of diarrhœa, it was of interest to observe its effect on the exposed colon. The dogs were injected with ½ c.c. of 5 p.c. morphine, and an hour later showed the customary symptoms, such as vomiting, defæcation and weakness of the hind legs. Observations made during the course of the day showed that the cæcum and ileo-cæcal region were the only portions of the gut to show any movement, and even these were very slight and occurred only occasionally. Very rarely a slight kneading movement appeared in the colon. Late in the day the colon became very distended. In no case was there any sign of anti-peristalsis or progressive movement in the colon. In one of our dogs ("Florence") the morphine seemed to cause a slight increase in the tone of the colon, an observation quite in accord with the recent work of Gruber and Robinson [1929] on the small intestine. This increase in tone probably accounts for the more frequent kneading movements in the dog.

Fig. 4 A and B are graphs of the observations made on the exposed colon after the injection of morphine.

Before any theoretical statement concerning the significance of these different movements in the dog's colon and cæcum is given, it may be said that the existence of anti-peristaltic waves in the dog's colon has hitherto been a subject of controversy. Cannon [1903] and Henderson [1909] are of the opinion that they do occur in the colon of the dog as well as the cat. Thomas and Kuntz [1926] also report a case in which they observed anti-peristaltic waves in the colon of a dog after giving large doses of nicotine. Elliott and Barclay-Smith [1904], although agreeing with Cannon concerning their appearance in the cat and other animals, were unable to detect any in dogs. Recently, however, Templeton and Lawson [1931], using the balloon method, recorded movements very similar to ours, and stated that they had found forward and backward movements passing along the colon of the dog, but hesitated to classify them as peristaltic and anti-peristaltic waves.

Just why there has been so much difficulty in finding these anti-peristaltic waves in the dog is hard to understand, since in our experiments we seldom failed to find them if the dog was observed for more than 20 minutes at a time. Furthermore, our observations on the ordinary cycle of movements in the colon of the dog are almost identical with those of Cannon on the cat, even in the rate of the waves and the

frequency of their appearance. What he refers to as a "marked bulging between successive constrictions following the deeper anti-peristaltic waves" we have called a "kneading" type of movement.

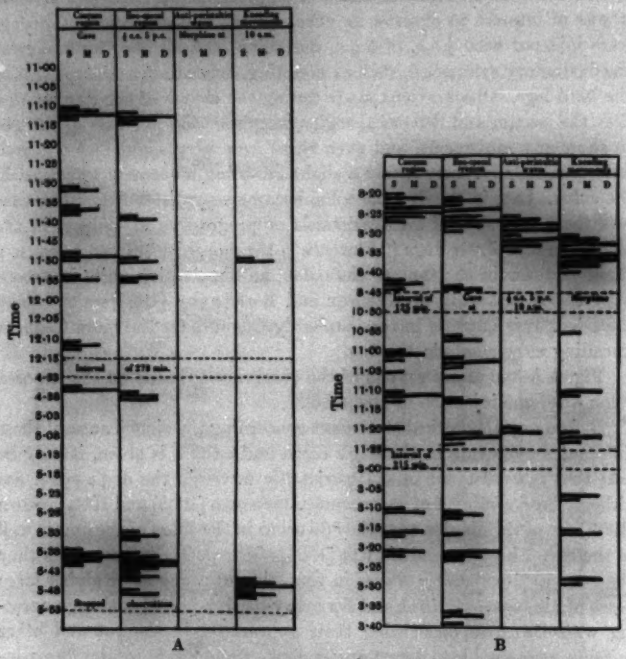

Fig. 4 A and B. The effects of morphine sulphate on the movements of the cæcum and colon at different intervals of time after subcutaneous injection.

The conclusions concerning the physiological significance of the movements we observed can be only theoretical and subject to criticism. But, judging from the consistency of their mode of action and the order of their appearance, we believe that the cæcum plays an important rôle in the concentration of the fæces. It also seems to be one of the functions of the colon, through its anti-peristaltic and kneading contractions, to churn and force fæcal material back toward the cæcum. The deep, slow, progressive wave along the colon, which has similarly been described by

Elliott and Barclay-Smith, we believe is the means of pushing the more concentrated material on down into the more distal colon, since it appears only when the material is quite concentrated, and was rarely noticed when the colon was distended with watery fæces after injections of $MgSO_4$.

Summary.

1. From 2 to 3 weeks after the colon and cæcum had been exteriorized, the water content of fæces began to diminish along with the return of activity and tone of the exteriorized parts.

2. There is a typical series of movements, consisting of cæcal contractions, followed by anti-peristaltic waves and then a type of kneading contractions in the colon. These series usually occur at intervals of about 30 minutes.

3. There is evidence of an occasional slow, deep, progressive wave in the colon.

4. We found anti-peristaltic waves in the colon of the dog, appearing at the rate of five and seven a minute.

5. There is evidence of a gastro-colic reflex, appearing within a few minutes after feeding.

6. Movements were observed after the administration of magnesium sulphate and morphine, which differed from the normal in both frequency and intensity.

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612.146:612.59

THE OPTIMUM TEMPERATURE FOR INVESTIGATIONS ON THE FROG'S CIRCULATION.

By E. M. SCARBOROUGH.

(*From the London (R.F.H.) School of Medicine for Women.*)

In a previous paper [Cullis and Scarborough, 1932] attention has been drawn to the influence of temperature in modifying the effects of certain drugs on the frog's circulation. Preliminary experiments on the influence of variation in temperature only on the blood-pressure, heart rate and web circulation of the frog were also reported. Before proceeding to further experiments involving the use of drugs, it seemed advisable to repeat a series of experiments in which, as far as possible, temperature should be the only variable factor, in order to ascertain the optimum temperature for the investigation of drug effects on the circulation.

Method.

The experiments were carried out during the winter months (November to March, 1931–2). With a few exceptions male frogs of fairly uniform size were selected. A method which has been previously described [Cullis and Scarborough, 1932] was used, and observations were made on the blood-pressure, heart rate and web circulation, the temperature being kept constant throughout each experiment. The series consisted of five groups of twenty consecutive experiments. The temperature of each group was different. The temperatures used were 5, 10, 15, 20 and 25° C.

Results.

The duration of most of the experiments was 3 to 4 hours. The following results are from observations taken at intervals of $\frac{1}{4}$ hour during the first 3 hours of each experiment. One experiment at 25° C. is omitted as the circulation failed within an hour.

The relationship between heart rate and temperature, which has been shown to be a linear one [Taylor, 1930], was maintained throughout

the series with three exceptions. These experiments (two at 20° C. and one at 25° C.) are also omitted. In each group the average heart rate at the end of 1 hour differed by less than 1 beat from the average rate at the end of 3 hours.

The relationship between blood-pressure and temperature was less obvious. The initial readings and the subsequent changes in blood-pressure varied considerably, and average figures do not fairly represent these variations. Fig. 1 shows the blood-pressure changes in five single experiments, one from each group, the experiment chosen being the one

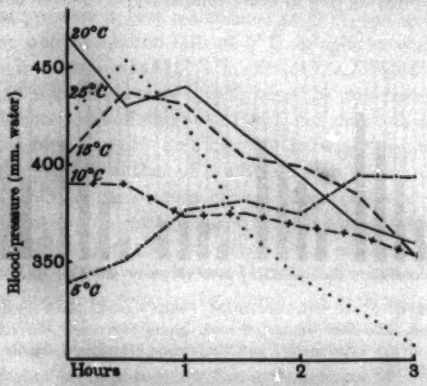

Fig. 1. Blood-pressure changes in single experiments at 5° C., 10° C., 15° C., 20° C., and 25° C.

which started nearest to the mean initial blood-pressure of the group. At 5° C. the blood-pressure rose during the experiments, with only two exceptions in which it fell. At higher temperatures the changes were more variable, but the blood-pressure showed a general tendency to fall throughout (at 10° C. there were two exceptions, at 15° C. seven, at 20° C. five and at 25° C. two in which the pressure was maintained or rose). The fall was most marked at 25° C. The initial readings were generally lowest at 5° C., and the final readings lowest at 25° C.

The web circulation was brisk and open (using "open" in reference to the number, not the calibre, of active vessels) at the onset of the majority of the experiments (80 p.c. or more at 5° C. to 20° C., 55 p.c.

at 25° C.). At the end of more than half of the experiments the web circulation was slow and closed (*i.e.* number of active vessels diminished). Fig. 2 gives a graphic representation of the condition in each group at hourly intervals throughout the series.

It will be seen that the web circulation failed most markedly at extreme temperatures, and only at 15° C. was well maintained for 3 hours. The web circulation was good in 72 p.c. of the observations in which the blood-pressure was above 350 mm. water and in 40 p.c. of those below. At 5° C. only a rise in blood-pressure was generally associated with impairment of the web circulation. In the few experiments in which blood-pressure rose at other temperatures the web circulation was

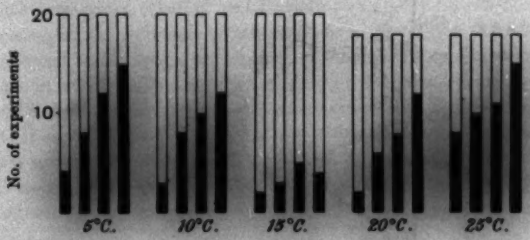

Fig. 2. Condition of the web circulation (white = good, black = poor) at different temperatures. The first column of each group represents the condition at the beginning of the experiments, and the second, third and fourth at the end of intervals of 1 hour.

maintained. At 15° C. a fall in blood-pressure was associated with a good web circulation, with two exceptions in which the fall was excessive. In experiments in which the blood-pressure fell at other temperatures there was a general tendency towards impairment of the web circulation. It is perhaps of interest to report that during the four experiments at 5° C. in which the web circulation was maintained the frogs struggled slightly. Such struggles occurred during three other experiments at 5° C. and occasionally at other temperatures. Throughout the series impairment of web circulation was associated with closing of the capillary network and slowing of the flow through the larger vessels. There was no marked change in the calibre of the active vessels, and no obvious difference between the impairment which developed at 5° C. and at 25° C.

The following results were obtained from experiments in which the frog's vessels were perfused with Ringer's solution at 15° C. and then at 5° C. or 25° C. At 5° C. the vessels were constricted in four out of six experiments; at 25° C. the vessels were dilated in four out of six. Direct application of water at 5° C. to the web caused closing of the capillary network in each of six observations, with slowing and congestion in the larger vessels in five out of six. Water at 25° C. caused transitory closing of the capillary network in three out of six observations, and no change in the larger vessels.

Discussion.

The blood-pressure is best maintained at 5° C., the web circulation at 15° C. The web circulation fails at 5° C. in spite of a rise in blood-pressure, and is maintained at 15° C. in spite of a fall. This result suggests that at 5° C. the blood-pressure is maintained or rises because the peripheral circulation is cut off. Brooks [1918] has shown that the leopard frog becomes very sluggish and inactive in water below 5° C. This inactivity, comparable to that of the hibernation period, may be associated with a similar circulatory change. The results of perfusion of vessels and direct application to the web indicate some active constriction at low temperature. Krogh [1920] found that local cooling produced dilatation in the lingual vessels, but [1921] that cutaneous capillaries differed from lingual in response to stimuli. At high temperatures the condition of the web circulation is largely determined by the actual height of the blood-pressure, and by the rate of fall.

The optimum temperature for the investigation of drug effects on various factors of the intact circulation is considered to be 15° C., given that occasional experiments are excluded in which the blood-pressure falls excessively and the web circulation is impaired. 15° C. is the temperature of the animal house in which the frogs are kept, and it is found that better results are obtained if the animals are acclimatized there for a few days before use. Further investigations are at present being made upon the effect of temperature on the absorption of drugs, and also upon the actual rate of absorption from the lymph spaces at 15° C.

Summary.

1. Results of experiments upon the influence of temperature on the circulation of the frog show (*a*) that the blood-pressure rises at 5° C., and tends to fall at other temperatures; (*b*) that an active web circulation is maintained best at 15° C.

2. It is suggested that the rise of blood-pressure at 5° C. is associated with an active constriction of the peripheral circulation.

3. 15° C. is considered to be the optimum temperature for the investigation of drug effects on the circulation.

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612.814:547.472.3

THE RÔLE OF LACTIC ACID IN NERVE ACTIVITY.

By T. P. FENG[1].

(From the Department of Physiology and Biochemistry, University College, London.)

GERARD AND MEYERHOF [1927] showed that nerve left in nitrogen produces lactic acid at a rate apparently unaffected by stimulation, and that the lactic acid formed during anaerobiosis is not removed on readmission of oxygen. Later Holmes, Gerard and Solomon [1930] found a slight rise in the lactic acid content of stimulated bull frog sciatics in oxygen, which, however, as the authors themselves pointed out, might really have been due to partial asphyxia on account of the close packing of the nerves. At any rate their parallel experiments on rabbits' nerves gave a slight decrease in the lactic acid content of stimulated ones. With this absence of a change in the lactic acid content there was also no change, as a result of stimulation, in the "free sugar" and glycogen. These results contrast strikingly with those found in muscle and make it doubtful whether lactic acid formation and oxidation constitute any part of the chemical events accompanying nerve activity. If, however, lactic acid forms no part of the working mechanism of nerve, it should make no immediate difference to the nerve whether its formation is suppressed or not. Poisoning, therefore, with iodoacetic acid furnishes a means of investigating the problem further.

The effect of iodoacetic acid was first roughly tested by soaking the nerve of a gastrocnemius-sciatic preparation in 1 p.c. iodoacetic acid (hereafter I.A.A.) with the muscle hung above the solution. After over 4 hours, crushing the nerve still called forth contraction. This at first gave the impression that nerve was practically immune to the action of I.A.A. The poisonous effect, however, was exposed by a study of the capacity of I.A.A. treated nerves for prolonged activity, employing the action current as indicator. Such nerves deteriorate progressively when tetanized and fail altogether in the course of 2 hours or so, depending on the degree of poisoning and the rate of stimulation.

[1] Tsing Hua University Fellow.

Method.

The sciatic nerves of medium-sized Hungarian *Rana esc.* were used throughout this work. In any one experiment two symmetrical nerves were employed, one serving as a control for the other. They were first soaked in the solution to be studied and then mounted in the ebonite chamber sketched in Fig. 1. The chamber was separated into two compartments (left and right, Fig. 1) by a wall provided with two slots, which accommodated the nerves and could be sealed when desired by vaseline. Each compartment was divided down the middle by an incomplete partition represented by the dotted line in Fig. 1. This parti-

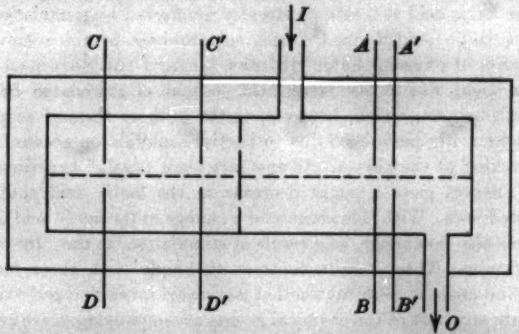

Fig. 1. Ebonite nerve chamber. *I*, gas inlet; *O*, gas outlet; *AA'*, *BB'*, stimulating electrodes; *CC'*, *DD'*, action current electrodes. See text for further description.

tion increased the surface for holding wet filter paper so that the nerves could be more easily kept moist, and helped to avoid electric leaks from one set of electrodes to the other. Electric insulation was further secured by coating the interior of the whole chamber with paraffin wax.

AA' and *BB'* are stimulating electrodes; *CC'* and *DD'* are leads to a Downing galvanometer with coils of total resistance about 20,000 ohms, the resistance of the nerve between the galvanometer leads being of the order of 70,000–80,000 ohms. The working sensitivity was about 1 mm. $= 5 \times 10^{-11}$ amp. A condenser of 20 mfd. capacity was in series with the galvanometer to block the injury current. Continuous stimulation was given in most experiments by an induction coil with a rate of about 50 make and 50 break shocks per sec., sometimes by repetitive condenser charge and discharge with the aid of a commutator. Test stimuli

were always by induction coil, tetanizing for a time sufficient for the galvanometer deflection just to reach its maximum, usually about 1 sec.

Apart from chemical determination of lactic acid formation there is no known reliable indicator of a sufficient degree of I.A.A. poisoning in nerve such as in muscle is afforded by the onset of contracture. After a number of trials concentrations around 0·4 p.c. were adopted as suitable. Such concentrations are well below that which may interfere with respiration [see Gerard, 1931] and give a striking poisonous effect to be described presently. Whether they inhibit the formation of lactic acid has not been determined chemically. It was found that for sure poisoning the duration of soaking must not be less than 2 hours. This is not astonishing since the connective tissue sheath of frog's nerve is a very efficient diffusion barrier [see Feng and Gerard, 1930], and I.A.A. poisoning is progressive, requiring some time to develop.

Results.

(1) *The rapid failure of* I.A.A. *poisoned nerve when stimulated in oxygen.*

The most conspicuous sign of I.A.A. poisoning in nerve is the inability of such nerve to endure prolonged activity. Fig. 2 plots galvanometer deflection, representing action current to a test stimulus, against time of tetanization. Curve *A* was given by a normal nerve; *B* by the symmetrical nerve previously soaked in 0·4 p.c. I.A.A. for 3 hours. The I.A.A. nerve failed completely after 1½ hours' continuous stimulation, while the normal nerve gave even a larger response after the same amount of stimulation. That failure is somehow brought about by stimulation and is not due merely to the gradual development of the I.A.A. effect is clearly shown by the steep fall of curve *B*, shortly after the application of continuous stimulation, from a slightly rising base line.

Assuming that with the concentration employed the above recorded effect of I.A.A. is entirely related to the inhibition of lactic acid formation, the rapid failure of stimulated I.A.A. nerve may be attributed (*a*) to the absence of formation of lactic acid with its attendant energy liberation, and (*b*) to the loss of the free energy that comes from the oxidation of the lactic acid formed. Probably both (*a*) and (*b*) are contributory to the deleterious effect of I.A.A., but they can be separately tested (i) by studying the performance of I.A.A. nerve in nitrogen and (ii) by supplying I.A.A. nerve with lactate.

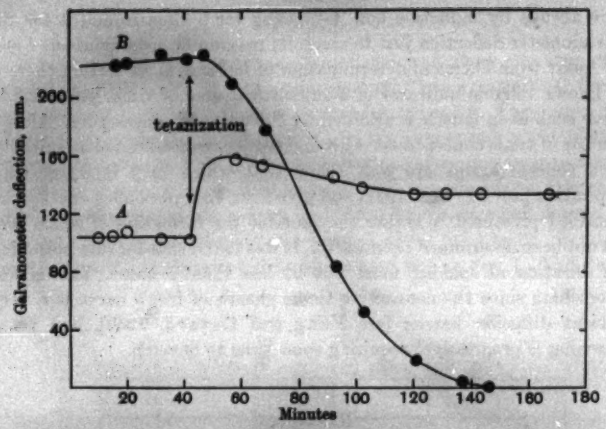

Fig. 2. The effect of continuous tetanization on the response: *A*, normal nerve; *B*, I.A.A. poisoned nerve: both in oxygen. The points plotted are galvanometer deflections representing action current to a test stimulus of about 1 sec. duration given by induction coil during a short gap in the tetanus. Note that *B* failed completely after about 100 min. tetanization, while *A* gave a greater response after the same amount of stimulation.

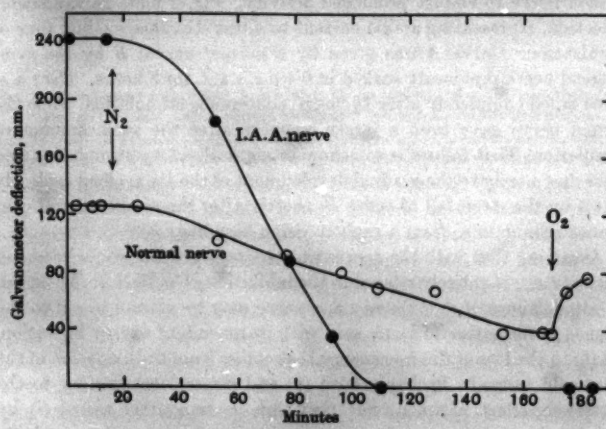

Fig. 3. The rate of asphyxiation of normal and I.A.A. poisoned nerve. Action current response to a short tetanus.

(2) *The greater susceptibility of* I.A.A. *nerve to oxygen want.*

Fig. 3 is one of four experiments on the rate of asphyxiation of normal and I.A.A. poisoned nerves which gave similar results. In such experiments frequent stimulation must be avoided because of the injurious effect of stimulation itself on I.A.A. nerve. In Fig. 3 the points plotted include all the test stimuli given, each being about 1 sec. tetanus by the induction coil. The poisoned nerve was completely asphyxiated in 1½ hours, while the normal nerve still gave considerable response after 2½ hours. The ratio of the times required to asphyxiate normal and I.A.A. nerves has not been determined exactly: approximating, however, it may be estimated that nerve poisoned with the concentration of I.A.A. used is asphyxiated in about one-third the time required for normal nerve.

One point to be particularly noted in Fig. 3 is the absence of recovery of I.A.A. nerve on readmission of oxygen. In the particular experiment shown oxygen was admitted 1 hour after complete asphyxiation of I.A.A. nerve. If this had occurred immediately after its failure, as in some other experiments, a certain amount of recovery would have occurred (see Table II). Apparently some irreversible process goes on which, past a point, makes oxidative recovery impossible.

The asphyxiation of nerve is primarily due to the depletion of the oxidation reserve, possibly in part also to the accumulation of toxic metabolites if such there be. It may be that I.A.A. nerve uses its oxidation reserve less economically, or that the products of disturbed metabolism are more toxic. It seems more likely that the formation of lactic acid with its attendant energy liberation, since anaerobic production of lactic acid by nerve is an established fact, helps to keep the nerve in working condition.

(3) *The effect of sodium lactate on nerve poisoned by* I.A.A.

For testing this point a phosphate-buffered I.A.A. solution of any required concentration is made up and divided into two portions, to one of which sodium lactate is added, usually in an amount to make the solution finally contain 180 mg./100 c.c. of lactate. Two symmetrical nerves are then soaked respectively in the two solutions. After 2 hours or more soaking, both are mounted in the chamber and continuous stimulation of both carried out simultaneously. Eight experiments of this kind were made, which are summarized in Table I, the nerve which had been soaked in I.A.A. solution containing lactate being always found to stand a considerably longer period of stimulation than its symmetrical

TABLE I.

No. of exp.	I.A.A. poisoning: Concentration p.c.	I.A.A. poisoning: Duration of soaking hr. min.	Time to complete exhaustion. Nerve previously in: I.A.A. alone hr. min.	Time to complete exhaustion. Nerve previously in: I.A.A. + lactic acid hr. min.	Concentration of lactate added mg./100 c.c.	Remarks
1	0·4	3 15	1 10	2 30	90	
2	0·4	3 20	1 15	2 15	180	Stimulation by induction coil at about 50 makes and 50 breaks per sec.
3	0·37	3 0	2 30	3 30	150	
4	0·37	3 50	1 20	2+	150	
5	0·37	3 40	1 10	1 40+	150	
6	0·4	3 5	2 20	3 20+	180	The lactate nerve in 180 mg./100 c.c. lactate for 3 hrs. before being poisoned.
7	0·4	2 5	3 0	4 30+	180	Slow stimulation at about 7 per sec.
8	0·4	4 15	2 10	4 30+	180	

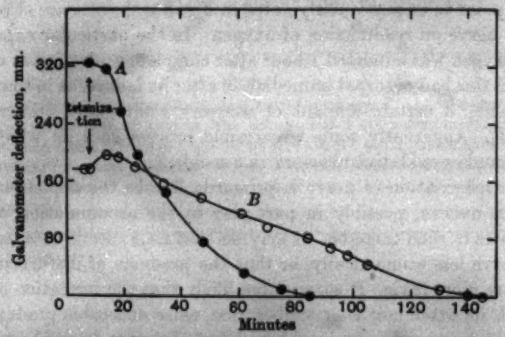

Fig. 4. The beneficial effect of sodium lactate on nerve poisoned by I.A.A. and continuously tetanized: *A*, by I.A.A. solution alone; *B*, by I.A.A. solution containing lactate in concentration 180 mg./100 c.c. Both in O_2. Response to a test stimulus during a short gap in the tetanus. Note that *B* endures tetanization much longer.

nerve poisoned in I.A.A. solution only. Fig. 4 shows one of these experiments, in which continuous stimulation was given by induction coil. The nerve soaked in I.A.A. alone failed completely after 1 hour 15 min., while it took 2 hours 15 min. to induce complete failure in the nerve treated with lactate as well as I.A.A.

There is no doubt about the beneficial effect of lactate on I.A.A. poisoned nerves in oxygen or air. In nitrogen, however, the effect is not obviously shown. Table II includes all the experiments made on this point. Of the eight experiments only two show a small difference in the time to complete asphyxiation in favour of the lactate nerve. On

TABLE II.

No. of exp.	Duration of previous soaking in I.A.A. solution hr. min.	Time to complete asphyxiation in min. Nerve previously in		Recovery on re-admission of oxygen: p.c. of initial deflection		Remarks
		I.A.A. alone	I.A.A. + lactic acid	I.A.A. alone	I.A.A. + lactic acid	
1	2 25	60	60	0	36	Nerve continuously stimulated in N_2
2	2 45	60	60	29	60	
3*	2 50	45	45	0	0	
4	4 10	100	110	0	12	Nerve resting in N_2
5	2 35	110	110	33	71	
6	2 50	160	160	29	91	
7	2 45	135	150	22	60	
8	6 15	160	160	9	19	

N.B. The concentration of I.A.A. used in this series of experiments was always 0·4 p.c. and that of lactate 180 mg./100 c.c.

* In this experiment oxygen was readmitted 40 min. after complete asphyxiation.

readmitting oxygen, however, the beneficial effect of lactate again manifested itself. The nerve containing lactate recovered more rapidly and more completely (Table II).

Thus experiments both in oxygen and nitrogen demonstrate that nerves with the ability to form lactic acid, or even containing added lactate, have a greater working capacity. One may conclude that nerve is capable of utilizing lactic acid by oxidation and that the formation of lactic acid is normally of functional importance.

A few miscellaneous observations were made. The poisonous effect of I.A.A. in nerve, as in muscle, is irreversible. A nerve first soaked in 0·4 p.c. I.A.A. solution for 2 hours and then transferred to normal Ringer for 3 hours behaves as if it had remained in I.A.A. solution all the time. Indeed the effect is progressive. Even without stimulation a poisoned nerve will become inexcitable after 10 hours or so. In this connection a curious phenomenon was found. A pair of symmetrical nerves was soaked in the same I.A.A. solution for 2 hours, one nerve was mounted in air, the other was allowed to remain in the poison solution; it is found that the one in air becomes inexcitable considerably earlier than that kept in the solution. This statement is based on two experiments. Possibly there are toxic metabolities which accumulate more in air than in solution.

DISCUSSION.

The present work shows on the one hand that the formation of lactic acid is not essential to nerve function, yet on the other that it is required if the nerve is to endure prolonged activity. This is the same as in muscle.

There, too, lactic acid formation, though not essential to contraction, is responsible for the normal capacity of a muscle to do a great amount of work, and as recently shown by Mawson [1932], an I.A.A. muscle receiving lactate can give a much greater number of contractions than one without. Such findings suggest that lactic acid in nerve, as in muscle, may be an intermediate state in the oxidation of carbohydrates.

The present work, showing the functional importance of lactic acid in nerve, and the chemical investigations, which have given no evidence for such importance, are not necessarily in conflict. If lactic acid is oxidized as soon as it is formed during nerve activity, there need not be any accumulation of lactic acid as a result of stimulation; the accumulation, if any, represents the difference between formation and removal, which would most probably be too small to be detected. Carbohydrate content, however, should be decreased by stimulation in any case, a point which chemical study has so far failed to reveal. The following calculation, however, is pertinent. When a nerve is stimulated continuously at any frequency higher than about 100 per sec., the heat rate reaches its maximum within half an hour and then progressively decreases. With a lower frequency of about 50 per sec., and employing stimuli with optimal characteristics, namely, properly timed condenser discharges, a steady state of heat production is usually established, at the rate of about 30×10^{-6} cal. per g. per sec. [Beresina and Feng, unpublished data]. Let us assume that during activity nerve burns glucose to CO_2 and H_2O, and let us take the heat of combustion of glucose to be 3·7 cal. per mg. Assuming all the energy liberated by stimulation to come ultimately from carbohydrate, the production of heat at the rate of 30×10^{-6} cal. per sec. for a period of 3 hours would require the consumption of only 0·09 mg. of glucose. This estimate is in all probability too high, and it would seem at least an open question whether the change in carbohydrate content due to stimulation falls within the limits of experimental error of present-day chemical methods.

Hill [1932] had very recently shown that the course of recovery heat production in nerve can be closely represented by a double exponential equation with a quick and small first term and a slow and large second term. Possibly the first term represented anaerobic recovery by lactic acid formation, while the second term was oxidative recovery. If such were the case, the heat production of I.A.A. poisoned nerve should follow a different course from normal. This had been put to experimental test. Fig. 5 shows the analysis of the heat production in an 8 sec. stimulus by I.A.A. poisoned nerves. Just as the action current of such nerves pro-

gressively becomes smaller after increasing amount of stimulation, their heat production also decreases very rapidly in the course of an experiment. In the experiment shown, which is the better of two made, twenty 8 sec. stimuli were given at regular intervals of about 5 min. The response to the last stimulus was only about half as large as that to the first. Eight stimuli were about of the same size, and the analysis given is of the average of these only. Neither in absolute value nor in time course is the heat production of I.A.A. nerve significantly different from normal [see Hill, 1932].

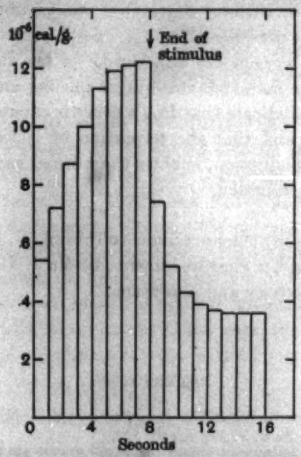

Fig. 5. Analysis of the heat production in an 8 sec. stimulus by I.A.A. poisoned nerve in O_2. It appears to be normal in shape.

The only previous investigation of which I am aware on the effect of I.A.A. on nerve activity is that of Ronzoni [1931], who reported that I.A.A. poisoned nerve stimulated twice every second is asphyxiated very much earlier in nitrogen than normal nerve similarly treated. The author, however, did not give any experimental details, not even the concentration of I.A.A. used, and it is difficult to appreciate fully the meaning of her results.

SUMMARY.

1. Frog's nerve is definitely poisoned by soaking in 0·37–0·4 p.c. iodoacetic acid for 2 hours or longer. The poisonous effect shows itself in the inability of such nerve to endure prolonged activity in oxygen.

2. Such nerve is also much more readily asphyxiated in nitrogen than normal.

3. Giving sodium lactate to poisoned nerve improves its capacity for prolonged activity in air or in oxygen; in nitrogen the time to complete asphyxiation, with or without continuous stimulation, does not seem to be lengthened by the presence of lactate, except occasionally and slightly. On readmission of oxygen the superiority of the poisoned nerve containing lactate again manifests itself in its quicker and greater recovery.

4. These results indicate that frog's nerve is capable of utilizing lactic acid by oxidation, and that the formation of lactic acid, though not essential to nerve conduction, enables the normal nerve to perform long hours of continuous function.

I wish to express my sincere thanks to Prof. A. V. Hill for his interest in this work and for his encouragement; to Mr J. L. Parkinson I am indebted for much advice and assistance.

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A COMPARISON OF THE PROPERTIES OF CERTAIN TISSUE EXTRACTS HAVING DEPRESSOR EFFECTS.

By RALPH H. MAJOR, J. B. NANNINGA and C. J. WEBER.

(From the Department of Internal Medicine, University of Kansas School of Medicine, Kansas City, Kansas.)

The number of recent papers on the depressor substances in the various tissues of the body reveals an increasing interest in the subject; Frey [1929], Lange [1930], v. Euler and Gaddum [1931], Zipf [1931], Drury and Szent-Györgi [1929], and Felix and Putzer-Reybegg [1932]. It seemed desirable to compare the extract with which we have been working [1929, 1930] with the substances obtained by these workers.

In our experiments we have used extracts of brain, liver, lung and pancreas, prepared in an identical manner, although more experiments were carried out with brain extract than with the other extracts. The experiments on blood-pressure were carried out under light ether anæsthesia.

Methods of preparation of the extracts.

Ten kg. of brain, liver, lung or pancreas were mixed with 10 l. of acetone and the mixture stirred vigorously for a period of an hour. After filtration the filtrate was evaporated to dryness and taken up with 200 c.c. of distilled water. This preparation will be referred to in the future as the crude extract of brain, liver, etc.

Crude extracts were thus obtained from various portions of the brain, and Fig. 1 shows that extracts from the basal ganglia are more active than extracts from certain other parts of the brain.

The effect of the various crude extracts after treatment with "norit" is shown in Fig. 2. This treatment, shaking up 25 c.c. of crude extract with 2 g. of norit, removes all the activity of the lung extract, 90 p.c. of the activity from the liver extract, 50 p.c. from the pancreas extract and only a small amount from the brain extract. Since this treatment removes histamine this is strong evidence that the activity of the lung

extract and most of the activity of the liver extract is due to histamine or histamine-like substances, as was shown by **Best, Dale, Dudley and Thorpe** [1927], and that the activity of the brain extract is not due to histamine.

Fig. 1. Curves showing the depressor effect of extracts from various parts of the brain. 1 c.c. of extract represents 0·5 g. of brain tissue.

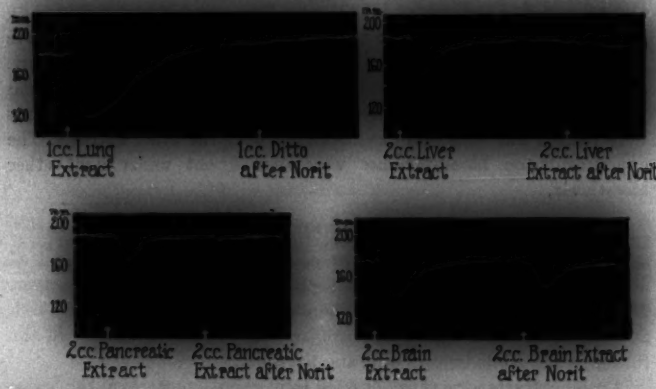

Fig. 2. The effect of treating the extracts with norit. Norit removes all of the depressor activity of the lung extract and most of the activity from extracts of liver and pancreas. Brain extract is only slightly affected by this treatment.

The behaviour of the crude extract after treatment with phosphotungstic acid is shown in Fig. 3. Treatment with phosphotungstic acid removes approximately 95 p.c. of the activity of the liver extract, 25 to 50 p.c. of that of the pancreatic extract, 95 p.c. of that of the lung extract and approximately 50 p.c. of that of the brain extract.

Phosphotungstic acid should remove histamine and choline and so evidence is furnished that the activity of brain extract is not due to these substances. Felix and Putzer-Reybegg, by fractional precipitation

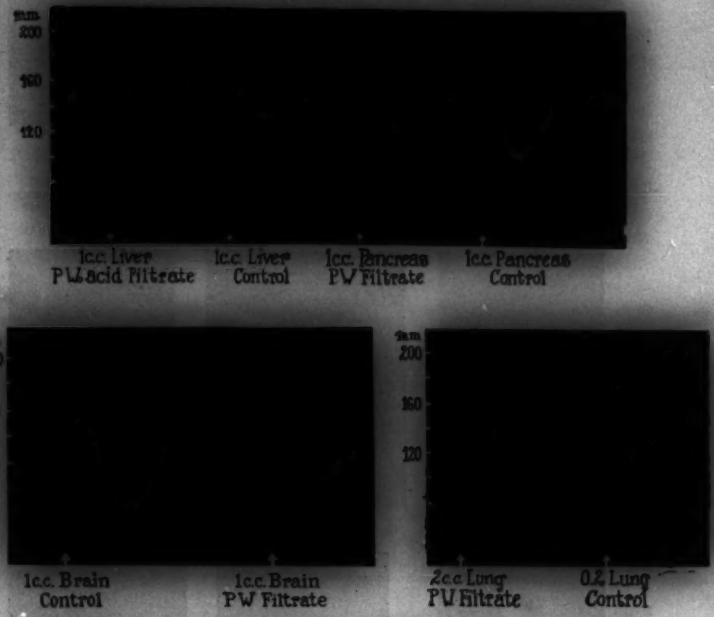

Fig. 3. The effect of treating the extracts with phosphotungstic acid. This treatment removes 95 p.c. of the depressor activity of the liver extract, 50 p.c. of the activity of the pancreatic extract, practically all of the activity of lung extract and less than 50 p.c. of the activity from the brain extract. Note that the amount of the lung extract control was only one-tenth of that used after treatment with phosphotungstic acid.

with silver, have brought evidence to show that 50 p.c. of the depressor activity of the precipitates from the kidney and mesentery was due to choline. Fig. 4 shows the effects of choline and of a silver filtrate of the brain extract, and that atropine abolishes the action of choline but has no effect upon that of the brain filtrate.

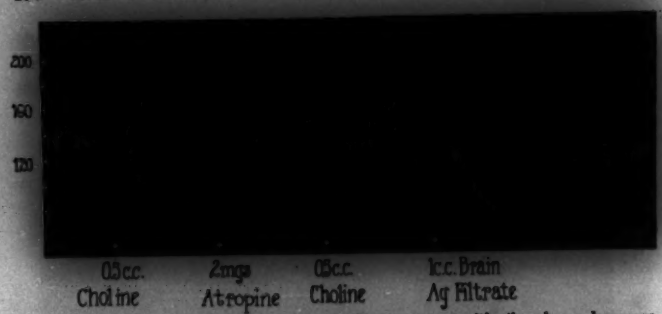

Fig. 4. Curve showing that the brain extract after treatment with silver has a depressor effect upon an atropinized animal.

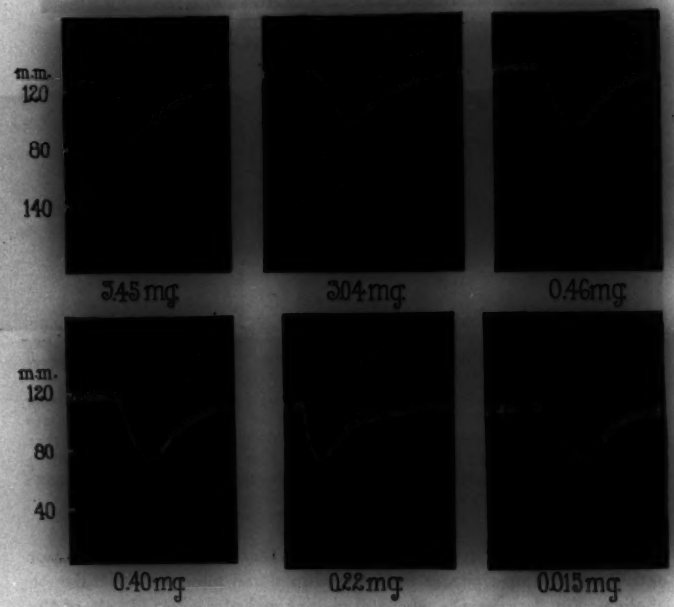

Fig. 5. Tracings showing purification of brain extract without loss of activity. Figures in terms of mg. of solids injected. The curve labelled 0·015 mg. shows the depressor effect of 0·015 mg. of histamine.

For the purification of the brain extract we have also proceeded as follows: the crude brain extract is treated with a saturated alcoholic solution of mercuric chloride and made alkaline; the abundant precipitate after removal of mercury can be divided into two fractions, one soluble in water, the other not; the active depressor principle with which we are working is in the former.

Fig. 5 shows the effects of purification upon the activity of the extract. The first curve shows the activity of the mercuric precipitate after removal of the mercury. The dose employed contained 3·40 mg. of solids. After treatment with phosphotungstic acid this extract shows the same activity when a dose containing 3·04 mg. of solids was injected. This solution was then made acid to Congo Red and treated with Lloyd's reagent, filtered, and the precipitate ground with barium hydroxide, water added, and again filtered. The barium was removed from the filtrate with sulphuric acid. The activity of an amount of the resulting solution which contained 0·46 mg. of solids is shown. When this solution was made alkaline and treated with norit, an amount of the filtrate containing 0·4 mg. of solids exhibits the depressor effect shown in the curve. This solution was evaporated to dryness and extracted with 90 p.c. alcohol, the alcoholic solution evaporated and taken up in water; an amount containing 0·22 mg. of solids has the activity shown in the curve, which is approximately equivalent to that produced by 0·015 mg. of histamine in aqueous solution.

We have also pharmacological evidence that these extracts do not owe their activity to either histamine or choline. In Fig. 6 the activity of the extract purified by treatment with Lloyd's reagent and alcohol is tested against a solution of histamine. The quantity of brain extract employed had about six times the depressor activity of the histamine used when tested on a dog under ether anæsthesia. The curve shows that the brain extract has no effect upon a virgin guinea-pig uterus, while the solution of histamine produced its characteristic response. This purified brain extract was next tested against the isolated intestinal loop of a rabbit. The intestinal loop showed the greatest response to choline, less to histamine, and showed no response whatever to the brain extract (Fig. 7).

Table I presents a comparison between the brain extract with which we have worked and the depressor substance described by other investigators. The "hormone" of Frey was isolated from the urine, the depressor substance of Felix and Lange was isolated from several organs, particularly the kidneys and the mesentery, while the depressor

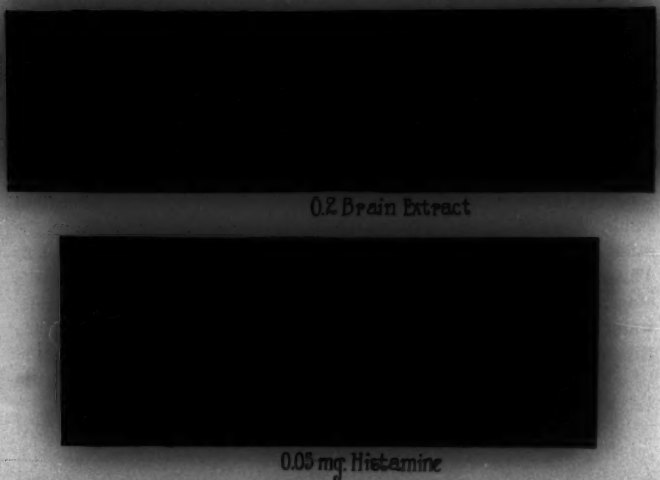

Fig. 6. Curve showing that the purified brain extract has no effect upon the virgin guinea-pig uterus, while histamine produces a characteristic contraction.

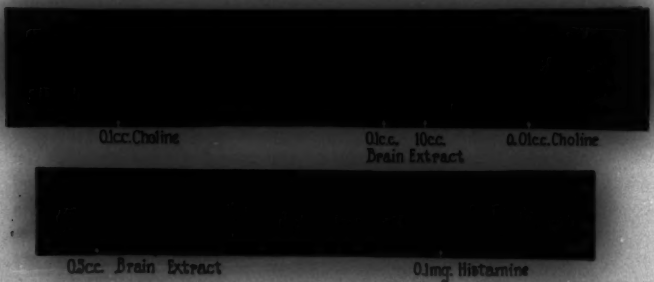

Fig. 7. Curve showing that the purified brain extract has no effect upon the isolated intestinal loop of the rabbit, while choline produces a marked contraction and histamine a lesser contraction.

extract of Euler and Gaddum was also obtained from several organs, extracts from the small intestine and brain apparently containing the largest amounts of the depressor principle.

TABLE I.

Treatment	Histamine	Choline	Adenosine	Frey's hormone	Euler and Gaddum	Felix and Lange	Brain
Silver precipitation	–	+	–	.	.	–	+
Phosphotungstic acid precipitation	–	–	–	–	–	–	+
Norit absorption in alkaline solution	–	.	.	.	.	.	+
Mercury precipitation in alcoholic solution (acid)	–	.	.	.	.	–	+
Mercury precipitation in alcoholic solution (alkaline)	–	–	–	.	–	–	–
H_2SO_4 5 p.c. Boiling 5 minutes	+	+	–	–	.	+	+
NaOH *N*/1. Boiling 5 minutes	+	.	+	–	–	.	+

+ or – indicates presence or absence of activity in the filtrate after indicated treatment

If the data given by these other observers are constant, we are apparently working with a different depressor substance.

SUMMARY.

The extract of brain tissue with which we have been working has a powerful effect. It is not precipitated by silver, phosphotungstic acid or mercury in acid alcoholic solution. It is not absorbed by norit from an aqueous solution, but is precipitated by mercury in alkaline alcoholic solution. It is not destroyed by boiling in 5 p.c. sulphuric acid or in normal sodium hydroxide for 5 minutes. It is active in atropinized animals and does not show a Pauly reaction. In one of our previous communications the statement was made that the purest solution we had obtained which contained this depressor principle showed a positive Sakaguchi reaction, indicating the presence of a guanidine compound. Since the publication of that paper we have obtained an extract which is quite active but gives a negative Sakaguchi reaction.

This work was aided by a grant from the National Research Council and the American Medical Association.

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PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
July 2, 1932.

Isolation of components in the retinal action potential of the decerebrate dark-adapted cat. By RAGNAR GRANIT.

Leads from the cornea and decerebration wound have been taken to the input of a directly coupled amplifier with a string galvanometer in the bridge formed by two valves working in a "push pull" arrangement. The aim of the work has been to try to establish a biological solution of the well-known complex action potential of the retina. In view of the high degree of differentiation of the cells in the various layers of the retina, the plan adopted was to look for an agent capable of influencing some parts of the action potential without interfering with others. Ether was chosen first for various reasons and was found to be satisfactory.

Etherization of a preparation, stable for several hours, removes in three characteristic steps definite components of the response to stimulation with white light. These components are indicated in Fig. 1 by the Roman letters in the order of their disappearance. Process I (P I) disappears rapidly during progressive etherization and the fast components are left unchanged. It is essentially a high-intensity process. Thus at a certain early stage of anæsthesia the slow component may be minute or even absent at high intensities whereas the low-intensity action potential is almost or even completely unchanged. Therefore the slow so-called *c*-wave of the composite potential is not homogeneous (see Fig. 1). P II next becomes sluggish during continued anæsthesia and ultimately disappears. Finally only the negative P III is left, provided the intensity has been high enough to elicit a negative component. So far the changes are reversible. The last stage is a gradual disappearance of P III. The changes have then become irreversible.

Records from the optic nerve indicate that P II is the component chiefly responsible for the impulses. P III has not been found associated with the setting-up of impulses. P I *may* promote the discharge but appears to be much less effective than P II, if at all so. The optic nerve discharge does not cease on removal of P I. The nerve records were obtained with needle-electrodes [Adrian and Bronk] stuck into *foramen opticum*, the galvanometer serving as an integrator.

It has been possible to confirm the relative independence of the three components in experiments where for some reason or other certain components have been lacking from the beginning.

The analysis of the experimental evidence supports the conclusion that the three processes pictured in Fig. 1 are the normal components of the action potential of an animal possessing very few cones. The initial negativity (*a*-wave) and the off-effect (*d*-wave) are recognized to be small and inconstant in certain mammals including the cat. Their place in the solution of the composite effect can be seen in Fig. 1. The typical off-effect is a retardation in the drop of potential following the removal of the stimulus. It may then be visible in the record from the optic nerve.

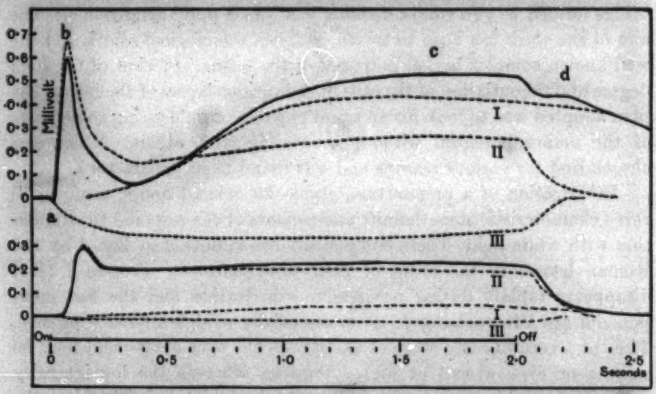

Fig. 1. Diagram showing solution of composite retinal action potential at two intensities of illumination in the ratio of 100 : 1. Broken lines: components. Lines drawn in full: action potentials as recorded; upper line in response to stimulus 100, lower line in response to stimulus 1. The *a*-wave is broadened slightly out of scale in order to show its derivation more clearly.

Observations on absence of cyanosis at great altitudes.

By Raymond Greene[1].

During the summer of 1931 I was a member of the expedition which made the ascent of Kamet, a mountain in the Himalayas 25,447 ft. in height. During the ascent I kept careful observation on the colour of the

[1] Schorstein Research Fellow, Oxford University. Medical Officer, British Himalayan Expedition, 1931.

lips and faces of my companions. We were all young, in good physical training, and well acclimatized to altitude.

Up to and including Camp V, which was pitched at a height of 23,300 ft., I observed no cyanosis, whether at rest or during exercise. Between this point and the summit I was, for the most part, climbing alone and therefore unable to observe. On the summit we encountered a strong and icy wind and we were all blue. But I do not think that we were bluer than we would have been under similar wind conditions at ordinary levels.

Barcroft and his colleagues found that natives of Cerro de Pasco (14,200 ft.) and Europeans who had lived there for a considerable time were distinctly blue. They report that members of the expedition were still blue after several days at this altitude, though less so than on arrival. On the other hand, after acclimatization members of the Pike's Peak Expedition were not blue, nor were those persons, such as the manager and the policeman, who had lived for three months on the summit. Members of the Pike's Peak Expedition did not become blue after short bouts of exercise. During long climbs cyanosis became very distinct, but it disappeared very rapidly on pausing.

Even during long climbs, however, I could detect no cyanosis in members of the Kamet Expedition. This was probably due to the fact that they were in far better training than members of the Pike's Peak Expedition, who were almost continuously occupied with laboratory experiments.

Release of extensor rigidity of the fore-limb by separation from lumbo-sacral segments. By T. C. Ruch. (*Oxford.*)

A post-brachial transection of the spinal cord which removes from the sphere of activity the lumbo-sacral segments augments many-fold the decerebrate rigidity in fore-limb extensors. The exaltation of rigidity is not due to irritation of ascending tracts by the cord lesion, for it occurs when the spinal cord has been severed weeks before examination. The phenomenon is considered an adorally acting "release of function" due to freeing the fore-limb reflex arcs from tonic inhibition originating below the level of section. *A priori*, such tonic inhibition would be expected to originate from the proprioceptive nerves from hind-limb muscles. But release of fore-limb extensor reflexes occurs when the portion of the cord removed has been previously deprived of a large share of its afferent paths by the nerve sections practised in preparation for reflex experiments on the hind-limbs. To follow out the implications

of this observation, the lumbo-sacral segments have been deafferented on both sides, decerebration performed, and, after recovery from the anæsthetic, the condition of fore-limb rigidity examined, either clinically in the intact limb, or by recourse to the optical isometric myograph and isolated triceps muscle. This more extensive reduction of the afferent paths which might act upon the reflex centres for the fore-limbs has been found in a series of experiments not to produce any detectable release. The stretch reflex of triceps muscle seems entirely normal in magnitude. When, however, transection is performed at the level of the highest spinal root severed, which eliminates only segments already deafferented, the usual exaltation of fore-limb rigidity occurs. Complete deafferentation is therefore not equivalent to cord section in freeing the fore-limb from inhibition. The ubiquitous possibility of excitation from the cut ends of spinal roots being the source of inhibitory impulses intrudes, although the data of Adrian [1930] suggest that the 20 hours allowed after deafferentation is ample time to insure complete freedom of the lumbo-sacral segments from afferent impulses. The conclusion reached is that a portion of spinal cord entirely deprived of centripetal impulses is still capable of restraining the activity of fore-limb proprioceptive reflex arcs. There appears to exist an adorally directed stream of inhibitory impulses of purely intracentral origin and such inhibition is, therefore, an automatic function of the spinal cord such as the automatic rhythmical functions under anæsthesia established by Graham Brown [1914].

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The dynamics of the ventricular contraction of the tortoise heart. By G. Stella. (*Univ. Coll. London.*) (*Preliminary communication.*)

The duration of the contraction of the ventricle is affected by a variety of conditions, among which the arterial resistance has long been recognized to play a considerable part. Thus O. Frank [1895] on the frog's heart, and De Heer [1912] and Patterson, Piper and Starling [1914] on the mammalian heart have observed that the higher the arterial resistance the longer the heart remains in a state of contraction. The mechanism, however, by which this affects the systole is not clear.

Experiments performed on the isolated ventricle of the tortoise heart

indicate that there is a tendency for the contractile stress set up in the heart muscle at any moment to be determined by its volume at that moment and by the time that has elapsed since the commencement of systole.

These were made at a low temperature (8°–10° C.) on ventricles driven artificially 6 to 10 beats per minute. The ventricle was perfused with a mixture of Ringer's solution and tortoise blood; changes in volume and in intra-ventricular pressure were recorded, the latter optically. The apparatus employed was similar to that used by Peserico [1928] modified so as to allow of changing the contraction from isotonic to isometric, and *vice versa*, at will. Under these conditions, and while the heart was working regularly and isotonically, input and output were cut off at the end of a systolic ejection so that the next systole should occur isometrically at the smallest volume reached by the preceding isotonic systole. The last part of the isometric contraction pressure curve was then found to coincide very well, within the limits of experimental error, with the last isometric part of the preceding isotonic systole; both curves, moreover, reached the zero line at about the same time from the beginning of the systole. Similar results were obtained with all arterial resistances investigated, and with very different volumes. In some experiments the systolic ejection was interrupted at different times from the beginning, so that the remaining part of the systole should occur isometrically, and the heart made to contract at the next systole isometrically at the volume it had at the time when the interruption was effected. Here, also, the second part of the two curves for intra-ventricular pressure showed a very satisfactory coincidence in course and time. All these facts are made clearer by the fact that variations in duration of systole for different volumes may be very considerable. The conclusion seems to be that the volume of the heart is a determining factor for the energy liberation not only at the moment of stimulation but throughout the whole systole. Quick release experiments were also performed by withdrawing a known amount of liquid from inside the heart at different moments from the beginning of an isometric systole. The fundamental findings described by Gasser and Hill [1924] on the redevelopment of tension for the striated muscle of the frog were found to repeat themselves here; according to Gasser and Hill, however, if the release takes place after the peak of the contraction, redevelopment does not occur. Here, however, redevelopment was found to take place even if the release was made as late as the second third of the downward part of the curve. Whenever the release was made not too abruptly the last part of the

contraction was found, moreover, to follow the course corresponding to the new volume.

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The effect of insulin on the relation between the oxidation and synthesis of sugar. By E. W. H. Cruickshank. (*Dept. of Physiology, Dalhousie University, Halifax, N.S.*) (*Preliminary communication.*)

The question as to whether or not insulin increases oxidative metabolism has been much debated. The discrepancy between results recorded has arisen from the difficulty in recording accurately the gaseous metabolism of a skeletal or cardiac muscle preparation and of estimating the changes in the glycogen content of the tissue under observation.

By an improved method for the estimation of oxygen and CO_2 it has been found that as far as the isolated heart is concerned hyperglycæmia produces a 10 p.c. increase in oxygen consumption with an equivalent increase in heat production, the respiratory quotient being unity. The addition of insulin (10 to 20 units of Iletin; Eli Lilly & Co.) in hyperglycæmia increases the oxidative changes by only 3 p.c., it alters the balance between oxidation and synthesis from a percentage normal ratio of 98 : 2 to one of 70 : 30, with the result that glycogen synthesis is increased some 30 p.c. Such a change is associated with a marked increase in the removal of sugar from the circulating blood. Similar experiments have been carried out with hearts subjected to a rapidly progressive hypoglycæmia, and the general trend of results is such that one is forced to the conclusion that, even with a rapid depletion of blood sugar, insulin still exerts a powerful synthetic action. In some hearts there is a distinct storing of glycogen, in others a definite sparing of carbohydrate stores. Oxidative processes may show the usual 3 p.c. increase or may suffer diminution. In conditions of hypoglycæmia where all the blood sugar used and a certain amount of muscle glycogen are oxidized, insulin changes the usual oxidation synthesis ratio from 100 : 0 to an average of 85 : 15.

Adrenaline and reflex excitability of the cardio-inhibitory centres. By G. Stella. (*Univ. Coll. London.*) (*Preliminary communication.*)

The question whether adrenaline has any effect upon the cardio-inhibitory centres remains still an open one since Oliver and Schäfer's [1894] first observations of a marked slowing of the heart rate following its intravenous injection.

Among recent workers, C. Heymans [1930] explains the bradycardia as a direct consequence of the primary rise of arterial pressure in the sensitive regions of the arch of aorta and carotid sinus; he points out, in fact, that if the depressor and carotid sinus nerves be cut, slowing of the heart rate from adrenaline is no longer observed (that is, if the dose of adrenaline be not too large). On the basis of such results Heymans emphatically denies that adrenaline in the usual doses has any effect upon the cardiac vagus centres.

On the other hand, experiments by independent perfusion of the head in the whole dog [Anrep and Starling, 1925], or in the innervated heart-lung preparation [Anrep and Segall, 1926; Volhard, 1930] have shown that adrenaline injected into the separate head circuit, and thus incapable of affecting directly the systemic arterial pressure, usually still causes slowing of the heart, both when the pressure in the carotid sinus is maintained artificially constant or after complete denervation of the latter, as in Volhard's experiments. The difference in the results of Heymans and those of Volhard is surmised by the former to be due to the abnormal conditions of the head perfused with defibrinated blood in the latter's experiments. It seemed, however, that a more fundamental difference between the two kinds of experiment lies in the fact that Heymans cut the afferent paths through which the arterial pressure regulates the heart-rate thus making it impossible to detect an action (if any) of adrenaline upon the responsiveness of the cardio-inhibitory centres to the afferent impulses from the aorta and carotid sinus.

The conclusion reached from the present investigation is that adrenaline actually does affect the reflex excitability of the cardio-inhibitory centres, in the sense that the degree of slowing of the heart produced reflexly by increasing the pressure in the carotid sinus or the arch of the aorta is far more pronounced when adrenaline is circulating in the head.

The experiments were performed on dogs anæsthetized by morphine (0·5 cg.) and urethane (0·3 g. per kg.); to study the carotid sinus reflexes, this was isolated and perfused on both sides with a technique

already described, and the depressor nerves were cut, leaving the vagi intact. It was found that after the intravenous injection of adrenaline (0·5–1 c.c. of 1:30,000 solution in a 10 kg. dog) a rise of the perfusion pressure in the carotid sinus was much more effective upon the heart rate than before the injection, and the effect coincided in time with the bradycardia observed in the intact animal. The same results were obtained by altering the pressure in the aorta in the innervated heart-lung preparation: in these experiments the head was perfused artificially by means of a Dixon pump, from the brachiocephalic, after careful denervation of both carotid sinuses. The slowing of the heart produced by increasing the systemic pressure was considerably more marked after injection of adrenaline in the circuit of the head. This was true also when, to counteract the effect of adrenaline vaso-constriction, the input from the pump was diminished so as to keep the pressure in the carotid arteries constant.

The marked bradycardia following an intravenous injection of adrenaline seems therefore to have as its cause the action of increased arterial pressure upon a more excitable cardio-inhibitory centre.

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Crystalline preparations with vitamin B_4 activity. By H. Barnes, J. R. O'Brien and V. B. Reader. (*Dept. of Biochemistry, Oxford.*) (*Preliminary communication.*)

A yield of 20–30 mg. of crystalline hydrochloride per cwt. of bakers' yeast has been obtained. The daily requirement of the adult rat is 0·01 mg. per day. (For method of assay see Reader [1930].) The preliminary extraction of the yeast and adsorption of the vitamin B_4 upon charcoal (*p*H 1) has been described elsewhere by Peters [1931]. This charcoal was extracted with 50 p.c. acid-alcohol, the alcohol removed *in vacuo*, and the extract subjected to successive treatments with mercuric sulphate, baryta, H_2S, and sodium phosphotungstate. A definite crystalline phosphotungstate, precipitated at *p*H 2, was obtained and recrystallized from 50 p.c. alcohol. It was then dissolved in 50 p.c. acetone to remove the phosphotungstic acid with baryta. The filtrate,

after removal of acetone, was hydrolysed by heating on the water bath for 1 hour with 5 p.c. HCl; then concentrated *in vacuo* to quite small volume (5 c.c. per cwt. original yeast). On addition of alcohol and ether and standing overnight crystals appeared. Fine needles, with M.P. 248° C. with charring. Preliminary analyses suggest the empirical formula of the anhydrous substance to be $C_4N_4H_5Cl$.

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The action of the vagus on the rhythm of the mammalian heart.

By G. L. Brown, J. C. Eccles and H. E. Hoff. (*Physiological Laboratory, Oxford.*)

Experiments have been performed with a view to investigating the effect of vagus stimulation on the rhythmic discharge of the nodal tissue of the mammalian heart.

The action potential of the sino-auricular node of the decerebrate cat was recorded with an amplifier and Matthews' oscillograph, as previously described by Eccles and Hoff [1931]. Single break induction shocks were applied to the peripheral ends of the right and left vagus through shielded electrodes in the neck.

A single volley so set up in either vagus effects a distinct slowing of the rhythm lasting not more than ½ min. The right vagus is usually more potent than the left. The effectiveness of the vagal volley upon that nodal cycle during which it falls depends upon its position in the cycle. For instance, the nodal cycle is not lengthened when the stimulus to the vagus is applied at a short interval before the next nodal discharge, though subsequent cycles are lengthened normally. This ineffective interval which follows the setting up of a vagal volley we have, for the following reasons, regarded as representing the true latent period of the vagal inhibition. Its value remains constant in any one animal, provided the temperature of the animal is constant. Its length appears to be independent of the duration of the nodal cycle, since it is not significantly changed by alterations in the length of the nodal cycle produced by the following means:

(*a*) Concurrent tetanic stimulation of the other vagus.

(*b*) Concurrent tetanic stimulation of the accelerantes.

(*c*) When the vagal stimulus is timed to fall during the lengthened (compensatory) cycle following an induced extra-systole.

The value of the latent period varies between 100σ and 160σ in various experiments and is the same for maximal and submaximal stimulation of the vagus.

The time course of the inhibition (Fig. 1, lower curve) shows a sharp maximum which follows so closely on the end of the latent period that it involves the first inhibited cycle. A second very flattened maximum may occur several cycles later.

The inhibition produced by a second volley, either in the same or opposite vagus, sums with that produced by the first. In Fig. 1, for example, the upper curve shows the time course of the inhibition produced by a second volley set up in the same vagus 390σ after the first. There is summation of the inhibitory effects produced by simultaneous stimulation of the opposite vagi.

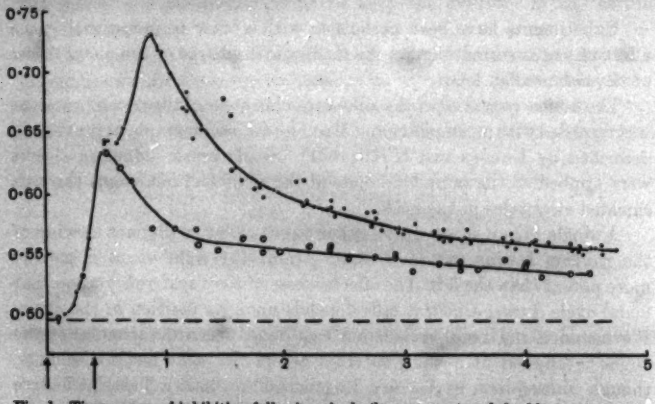

Fig. 1. Time course of inhibition following single (lower curve) and double (upper curve) stimuli applied to the right vagus. Times of the stimuli are shown by the arrows. Ordinates—duration in seconds of each nodal cycle. Abscissæ—interval in seconds between stimulus and end of corresponding cycle.

REFERENCE.

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Inhibition in peripheral preparations of skeletal muscle.

By GRACE BRISCOE.

In reflex preparations inhibition is demonstrated against a background of existing contraction, and shows itself as a loss or reduction of contractile tension. In peripheral preparations a sudden change in the rate of the stimulating current produces, under certain circumstances, a loss or reduction of existing tension, and this fall of tension may be more rapid than that due to cessation of stimulus.

The double reaction of reciprocal contraction and inhibition in antagonistic muscles can be shown if certain experimental conditions as to anæsthesia, differences in rate and strength of the stimulating currents, be observed.

If a muscle be maintained for a short period (*e.g.* 10–20 seconds) in a state of strong postural contraction by a suitable slow rate of stimulus applied to the peripheral nerve trunk, then a sudden change to a fast rate (*i.e.* one capable of carrying out phasic movements) will produce a sudden relaxation, although this same fast rate is of such a character that it would have produced a full contraction in the resting muscle. On the other hand, if a muscle be maintained in a condition of mild postural contraction, a sudden increase of rate of stimulation will augment the existing contraction. In other words, the result of a given stimulus is dependent on the receptive condition of the muscle concerned; if passive —contraction; if engaged in a different kind of functional activity— relaxation.

An extensor muscle is prepared in one limb and a flexor on the other side, so as to avoid interference. Neon lamp discharges are used as the source of stimulation [Briscoe and Leyshon, 1929]. The two nerves always receive the same rate of stimulation, whatever that rate may be. The strength of the stimulating currents is regulated by separate potential dividers, and is varied to either nerve according to the conditions of the experiment.

The neon lamp is made to flash at a slow *P* (postural) rate [Briscoe, 1931]. The strength of potential applied is so arranged that extensor develops a strong postural contraction, flexor a weak postural contraction. A sudden increase to an *M* (movement) rate now causes opposite effects in the antagonistic muscles. Extensor relaxes while flexor augments. Changing back to a *P* rate causes extensor to contract, while flexor relaxes. (See Fig. 1.)

If conditions as to strength of stimulus be reversed, so that flexor is placed in strong postural contraction, change to an *M* rate produces relaxation in the flexor and augmentation of contraction in the extensor. Similar muscles can be prepared, *e.g.* quadriceps in either limb, and made to show reciprocal contraction and inhibition.

It is improbable that these reactions take place in the nerve trunk at the site of stimulation, for they are affected by drugs introduced into the circulation. It is also improbable that they are confined to the neuromuscular junctions, because the same type of reaction can be demonstrated in muscles which are stimulated directly. The changes, however, are not so extensive as with indirect stimulation, probably because it is difficult to stimulate the whole muscle directly.

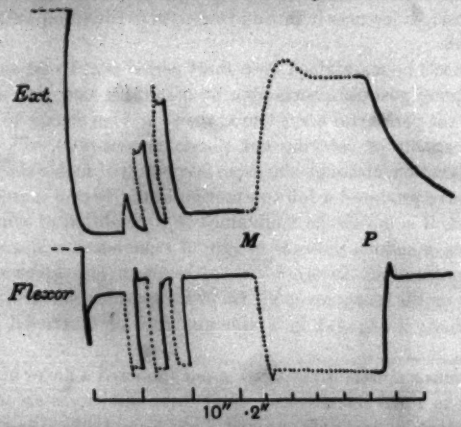

Fig. 1. Cat. Dial and ether. Tracing from record. Upper line right quadriceps, lower left tibialis and ext. l. dig. Contraction downwards. Whole line indicates when rate suitable for posture used (*P*), dotted line when rate suitable for movement used (*M*). Rates changed by plugging ½ megohm resistance in and out of charging circuit. No change in strength or character of stimuli. Extensor receives maximal, flexor submaximal *P* stimulus. First three spells of *M* stimuli recorded on slow drum, fourth on fast drum. Inhibitory effect not so marked in early stages of a posture.

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Normal gas tensions in the mucous membrane of the rabbit's uterus. By J. ARGYLL CAMPBELL. (*National Institute for Medical Research.*)

The following experiments were carried out for Dr Arthur Walton of the School of Agriculture, Cambridge, who requested information on gas tensions within the uterus.

Six rabbits (2–3 kg.) were anæsthetized with amytal and in addition a little A.C.E. mixture was required for three of them during the operation. In this the abdomen was opened for about 3 inches just above the symphysis pubis and small clips were placed at the free ends of the Fallopian tubes to close their openings into the abdominal cavity. A ligature was placed either at the internal or external end of the vagina. Up to 20 c.c. of oxygen and nitrogen gas mixtures, with oxygen tensions varying between 0 and 50 mm. Hg, were injected into the main cavity of the uterus by means of a hypodermic needle and gas container. After closing the abdomen, the lower half of the animal's body was kept under saline at 38 to 40° C. Samples (2 to 10 c.c.) of gas were withdrawn from the uterus usually at hourly intervals up to about the fifth hour after injection, and the oxygen and carbon dioxide tensions were calculated in the usual way after analysis in the small or large Haldane analyser. The results are recorded in Fig. 1, at zero hour, the tensions of the injected gases being shown. Anæsthetics as used in these experiments decrease the oxygen tension and increase the carbon dioxide tension on an average by 4 mm. Hg, so that allowance must be made for this. The numbers in Fig. 1 refer to the different experiments. Where the lines become horizontal, equilibrium between the gas tensions in the free gas in the uterine cavity and those in the cells of the mucous membrane has become established. Straight lines and not curves have been drawn between the points to distinguish the different experiments. Of course the carbon dioxide tension rises much more steeply than shown in most of the experiments.

The oxygen tension in five of the rabbits is obviously somewhere between 30 and 45 mm. Hg. In the remaining case, after allowing for effect of anæsthesia, the oxygen tension is about 20 mm. Hg, so that for all the rabbits investigated the limits for oxygen tensions are 20–45 mm. Hg.

In all six experiments the limits for carbon dioxide tensions are 40 and 60 mm. Hg. The point above 60 is probably due to anæsthesia.

These results are in general agreement with the figures for gas tensions in many other tissues of the body [Campbell, 1931].

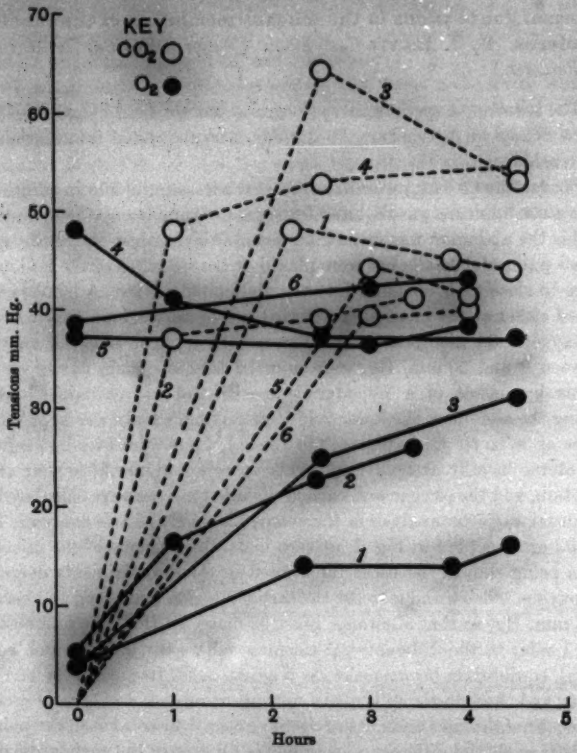

Fig. 1.

Similar results were obtained with the uterus of the cat, injecting only a bubble of gas, which was analysed in a Krogh micro-apparatus using 75–80 per cent. glycerol in water to enclose the bubble.

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Humoral transmission and the chorda tympani.

By O. S. Gibbs[1] and J. Szelöczey[2].

A considerable bulk of evidence has now accumulated to show that when a para-sympathetic nerve is stimulated a substance is liberated which possesses the pharmacological properties of acetyl choline, and which is probably the physiological connection between the nerve and its end organ. It is now certain that physostigmine acts by preventing the rapid normal destruction of this substance. This latter discovery makes it easy to demonstrate the production of the para-sympathetic hormone which we have been able to obtain in a relatively clean solution by a special method of perfusion from the submaxillary glands of both cat and dog. Essentially the experiment consists of perfusing the gland only for short periods during which the chorda tympani is stimulated, and immediately restoring the normal blood stream. After small quantities of physostigmine have been administered one may wash out from the gland during chorda stimulation a highly active substance. This substance we call "Chordastoff." Tested by injecting into the gland artery, "Chordastoff" shows itself to be a powerful secretory stimulant; on the blood-pressure it has a powerful depressor effect; to the isolated frog's heart it is depressant, and to the isolated rabbit's intestine, stimulant. In all four places this action is prevented by atropine, and both qualitatively and quantitatively it behaves like acetyl choline. Furthermore, the substance is rapidly destroyed by blood, or blood cells, which destruction is prevented by physostigmine. We are therefore able to confirm on the gland the work of Lœwi on the isolated frog's heart, and that of Engelhart on the eye. We also establish beyond any doubt the presence of this substance in the gland during stimulation of the chorda tympani.

These experiments were performed in the Pharmacological Institute, University of Vienna.

[1] Travelling Rockefeller Fellow of the Med. Res. Council.
[2] Stipendiat from the Hungarian Government.

The depressor constituents of muscle extracts. By A. N. Drury[1].
(*Department of Pathology, Cambridge.*)

If a neutralized trichloracetic acid extract of ox heart muscle is introduced intravenously into the rat the heart rate is temporarily slowed. This bradycardia appears to be an A.-V. rhythm, and sets in and ends abruptly. A similar disturbance is produced by injecting muscle

[1] Working on behalf of the Medical Research Council.

adenylic acid. By comparing the duration of the effect produced by the muscle extract with that produced by very small doses of muscle adenylic acid an estimate can be made of the amount of the responsible substance or substances. About 100 mg. of substance per 100 g. of fresh tissue are found to be present. On the other hand, the same muscle extract produces a heart block in the guinea-pig which has been shown to be due to adenylic acid [Drury and Szent-Györgyi, 1929; Bennet and Drury, 1931]. By comparing the effect in this animal with small doses of adenylic acid, the muscle extract contains only about 50 mg. of adenylic acid per 100 g. of fresh tissue.

The results suggest that a substance is present in addition to adenylic acid which has no influence upon the guinea-pig's heart and produces an A.-V. rhythm in the rat's heart.

The purification of the muscle extract on the lines already reported [Drury and Szent-Györgyi, 1929] shows that the barium salt of the additional substance is soluble in water while the lead salt is insoluble. As the same salts of adenylic acid have similar properties, it would appear that the additional substance is allied to adenylic acid. It is, however, less readily inactivated by hydrochloric acid than adenylic acid.

Cytidylic acid, derived from yeast nucleic acid, in doses of 2 mg. and under, does not disturb the rhythm of the guinea-pig's heart and produces an effect in the rat identical with that seen after introducing muscle adenylic acid. A mixture of pure adenylic acid and yeast cytidylic acid has the same characteristics as the muscle extract, for if it is compared in the guinea-pig with known doses of adenylic acid it is less active than when it is similarly compared in the rat.

As cytidylic acid is more resistant to mineral acids than adenylic acid, it would seem reasonable that this, or some closely allied substance, is the additional factor present.

Yeast cytidylic acid, introduced intravenously in doses of 1·0 mgm. into the rabbit, causes a fall in blood-pressure of about 30 mm. Hg. which is not abolished by atropine. From the above experiments it would appear probable that cytidylic acid is present in muscle extracts and plays a part in their depressor activity. It may be the depressor substance reported [Bennet and Drury, 1931] in the hydrolysate from the hydrolysis of yeast nucleic acid which was inactivated with difficulty by hydrochloric acid.

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Crystalline preparations of vitamin B_1 from baker's yeast. By H. W. Kinnersley, J. R. O'Brien and R. A. Peters. (*Dept. of Biochemistry, Oxford.*) (*Preliminary communication.*)

The charcoal adsorption methods [Kinnersley and Peters, 1929] have been applied upon a large scale to 2000 kg. yeast with recent improvements [Peters *et al.* 1932]. A crystalline hydrochloride of activity 0·002–0·004 mg. (2–4γ) per diem (pigeon dose) (≡ approx. 1·5 vitamin B_1 units) has been reached in yield of some 30 mg. per 50 kg. moist yeast. After extraction with dilute HCl of the vitamin B_1 adsorbed at *p*H 7·0 on the charcoal, phosphotungstate precipitation by the new technique precipitated all the vitamin between *p*H 5–2·0. Activity approximately 0·1 mg. By the successive stages of alcohol-phosphotungstate-alcohol, an activity of 0·005 mg. (5γ) is obtained in yield of 20–25,000 pigeon doses per 100 kilo. This stage is semi-crystalline. Precipitation by aqueous gold chloride, followed by crystallization from acid alcohol or acid alcohol-petrol ether mixtures gave readily needle-shaped crystals, which on recrystallization appear as clusters of irregular plates. There is some evidence that more active material is present. Specimens of Jansen and Donath crystals of vitamin B_1 from rice had activity 5–8γ by the same methods of test: the crystals from yeast therefore seem to be a purer source of vitamin B_1. If the sulphur containing crystalline substance from yeast of Windaus, Tschesche *et al.* [1931] is identical with Jansen and Donath's substance, as suggested by Tschesche [1932] and by van Ween [1932], then our product would be a purer preparation of vitamin B_1. The crystals melt at 227–230° C., with decomposition, and the picrolonate at 165° C. By the Pauly test (after Koessler and Hanke) 10γ gives a yellow colour. Sulphur is present in the final crystals and also before the gold stage. Since the method of preparation does not include treatment with H_2S before the gold stage this is suggestive proof that this sulphur is not introduced by the H_2S at the gold stage.

(We are much indebted to Dr B. C. P. Jansen for the specimen of crystals here used.)

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DIRECTIONS TO AUTHORS WITH REGARD TO THE PREPARATION OF PAPERS SUBMITTED FOR PUBLICATION IN THE *JOURNAL OF PHYSIOLOGY*

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DIRECTIONS TO AUTHORS WITH REGARD TO THE PREPARATION OF PAPERS SUBMITTED FOR PUBLICATION IN THE *JOURNAL OF PHYSIOLOGY*

Two difficulties in connection with the publication of scientific papers are of great and increasing importance, namely cost and bulk. It is to the interest of authors no less than of readers that both should be controlled, and it is hoped that careful attention to the following notes will help authors to prepare their papers in such a way as to save much unnecessary editorial work, diminish the probability of rejection and increase the appreciation of their work by those who read the *Journal*. It should be remembered that work badly presented will most probably be largely wasted, and that the completion of a concise and readable account of a research is the final stage of the research itself.

GENERAL DIRECTIONS

It is highly desirable, and very helpful to readers, that papers should be built up in accordance with a definite plan. This advantage is all the greater if the same general principles are always adhered to. It may be laid down therefore that all papers should state clearly and concisely in the following order: (1) the objects of the research, (2) the means used, (3) the results, (4) discussion and inferences, (5) a summary.

(1) In stating the object in view it is generally necessary to refer to previous work. Such references however must be confined strictly to those which are essential and must not be allowed to become a historical review.

(2) In describing the methods used authors should be on their guard against giving a verbal description in general terms and then repeating it in greater detail either verbally or in the form of needlessly elaborate protocols of experiments.

(3) In stating results it is often necessary to give tables of figures the meaning of which may be made clearer by means of graphs. There is however in this respect a danger of needless repetition and all possible care should be taken to avoid this. Further, when the results of a number of experiments of the same type have to be given, it is not necessary to describe more than one in full detail with tabular protocol, etc. It is better and sufficient to describe one experiment fully and to give only the results of the others with the statement that they were performed in the same way as the experiment recorded in full.

(4) Care should be taken to avoid obscurity in the statement of the results as a whole and in their discussion. Statement should, so far as possible, be distinct from discussion, and it should be remembered that clarity is by no means incompatible with brevity. In fact readers' impressions of an author are influenced greatly by his literary style. It is therefore in an author's own interest that he should take pains about the style of his writing. Sentences should be short and, generally speaking, a short word is to be preferred to a long word. It is usually better to use words of Anglo-Saxon origin in preference to those derived

from Latin or Greek. A single word should always be preferred to an involved circumlocution. For instance, expressions in the left-hand column below may often be replaced by those in the right-hand column:

a considerable time	long
admirably	well
appear	seem
attempt	try
commenced	began
considered	thought
courses	runs
it would appear questionable	it is doubtful
large number of	many
led to the provisional hypothesis	suggested
mode of procedure	method
on account of	because of
presenting a fresh communication on	discussing again
previously to	before
selected	chosen
subsequently to	after

Finally, authors should remember that papers which are mainly of clinical or pathological interest are not suitable for publication in the *Journal of Physiology*.

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Papers must be typewritten on one side of the paper only, and the typescript should be carefully revised and corrected by the author before it is sent in. MS. insertions and corrections should be avoided as far as possible, but where they are unavoidable particular care should be taken to write proper names and technical terms legibly.

Papers should be sent in packed flat and not rolled or folded.

Any matter which is to be printed in a different type from that used for the bulk of the paper, *e.g.* reference lists, tables, paragraphs in small type, etc., should be sent in on separate sheets of paper, if possible, uniform in size with that used for the main typescript. Such matter should be marked by a vertical line on the left-hand side to indicate that a different type is to be used.

Quotations should not be given unless essential and, if more than four lines long, should be treated as just indicated for matter in different type.

It is generally desirable that the choice of type, etc., *e.g.* cross-headings, should be left to the Press reader, but if an author has some special reason for wishing certain words to be printed differently from the main bulk of the paper, he may indicate his desire, and in that case the following conventions for instructions to the printer should be followed:

(1) One underline indicates italics.
Two underlines indicate small capitals
Three underlines indicate capitals.
A wavy line (~~~~~~) indicates clarendon type, thus **clarendon.**

(2) A vertical line on the left-hand side indicates that the part of the typescript so marked is to be printed in a type different from that used for the main body of the paper.

(3) Words which are meant as instructions to the printer and which are not to be part of the text should be enclosed in lines and brackets, *e.g.* (insert Table I).

(4) Paragraphs are indicated by indenting the first word a little further to the right.

(5) Capital letters and hyphens should be used as sparingly as possible.

(6) Cross-headings should be underlined twice and opposite each should be written in the margin of the typescript "sm. caps. cent." to indicate to the compositor that they are to be printed centrally in small capitals. If any headings are needed for subdivisions of these main sections they should be marked "ital. cent.," "ital. indent.," "r.o. = run on" according to their importance, and underlined in the appropriate way.

(7) All tables, figures and plates should be indicated as follows: Table I, Fig. 1, Pl. I. If more than one figure on plate: Pl. I, fig. 3.

It would greatly assist if the place where the above are required is marked on the side of MS.: viz. "Table I near here," and so on.

2. Proofs

Proofs are sent to authors together with their copy to enable them to correct mistakes in printing, *not* to enable them to make additions. It is important that the original copy sent in should be prepared carefully so that there may be no need for subsequent additions. If however any additions in the middle of a paragraph are unavoidable, an equal number of letters in the immediate vicinity should be deleted. If this is not done considerable rearrangement of the type already set up may be needed. For this reason additions in proof may be disallowed. There is somewhat less objection to adding a new paragraph than to making additions in the middle of an existing one.

Proof is often sent back with queries for the author to settle. A question-mark in ink at the side of the type calls attention to these, and the proof reader often adds a few words of explanation. As a rule the author will find that he has been inconsistent, that he has spelled the name of an author differently in the text and in the references at the end, given different initials or a different date, etc. Care should be taken to make the necessary correction or else to indicate that none is necessary.

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The conventions adopted for proof correcting are indicated in Appendix I.

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The title of the paper—as brief as possible—should be typed on a separate sheet of paper. On this sheet the following should also be typed below the title:

(1) The name of the author, or authors, followed, when necessary, by descriptions, *e.g.* (*Grocers' Company Research Scholar*). When a paper is by several authors the names should be given in alphabetical order.

(2) The place where the work was done, *e.g.* (*From the Department of Physiology, Cambridge*).

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When a paper appears in the *Journal of Physiology* it has a short title printed at the head of the right-hand pages. This title is used in the alphabetical index of the volume. It should be chosen with care and should convey the scope of the paper as far as possible, but must not exceed four or five words in length, as it is printed in capitals. The editors would be saved some trouble if authors would supply the short title themselves, either on the proof or on the original MS. In the latter case it should be headed "short title" and be on the same sheet as the title of the paper.

5. Table of Contents

A table of contents or synopsis is usually needless, since cross-headings to sections are adequate.

6. Cross-headings

A heading "Introduction" at the beginning of a paper is unnecessary.

Whenever possible papers should be divided into sections corresponding to the chief subdivisions of the work. These sections should have short headings descriptive of their contents.

7. Footnotes

Footnotes are both an expense and a curse and should be avoided. Descriptive or explanatory words can nearly always be embodied in the text without inconvenience. When the use of footnotes is absolutely unavoidable they should be numbered consecutively and placed at the foot of each folio of MS., corresponding numbers being inserted at the appropriate places in the text. The footnotes are placed in the proper position by the printer when the paper is paged.

8. Tables

The printing of tables is particularly expensive, they should therefore be as few as possible.

(1) Tables should be given on separate sheets, together with descriptive heading and any legend or explanatory wording which is really a part of the table. Such sheets should be numbered consecutively in the order in which the tables are to appear in the text. Corresponding instructions to the printer should be placed in appropriate positions in the text and must have a line drawn round them to indicate that they are merely instructions and not part of the text. It must be understood that the compositor must to some extent use his judgment about obeying such instructions.

References to tables in the text must give the appropriate numbers and such expressions as "the following table" must be avoided.

(2) Care should be taken to avoid needless detail in tables.

(3) Repetition in vertical columns should be avoided as far as possible. This can be done to a great extent by suitable choice of headings to columns.

(4) The number of "rules," especially vertical "rules," must be as small as possible.

9. Illustrations

Illustrations have to be reproduced photographically. They should therefore be kept quite separate from the text as they have to be sent to the engraver when the MS. is sent to the printer. Authors should indicate in the margin of the text the approximate position of the figures. It is also important that they should be numbered consecutively and bear the author's name, and that "legends"

or descriptive matter should be typed on separate sheets of paper and bear corresponding consecutive numbers. This is because the "legends" are set up in type by the printer and are not dealt with by the engraver. Magnifications should be stated exactly, *e.g.* × 100. Photographic reproduction of illustrations is carried out by means of "half-tone" blocks or "line" blocks. The former are more costly in themselves than the latter and have to be printed on special paper. This involves extra cost for paper, printing and binding. Illustrations should therefore be line drawings or tracings, not photographs or wash drawings. The illustrations sent in with a paper should be limited strictly to such as are essential for the elucidation of the text.

In preparing illustrations the following directions must be observed carefully:

(1) Illustrations must if possible be tracings or line drawings, not photographs.

(2) Line drawings must be in Indian ink on white or faintly blue-lined paper, or better, on Bristol board.

(3) Drawings should be about twice as large as the reproduction will be. Hence lines must be thick enough to show up clearly after reduction. It should be remembered that the greatest area available for an illustration and its legend is $6\frac{1}{4}'' \times 4\frac{1}{8}''$, but reproductions should not be larger than the minimum size necessary for clearness.

(4) Lettering is best left to the artist and should therefore not be in ink, but should be written *legibly* in pencil on the graph. Care should be taken about the accuracy of the lettering since the draughtsman will follow it exactly.

(5) No change can be made in a block once it has been prepared. Great care therefore should be taken about the accuracy of illustrations.

(6) Tracings for reproduction should be taken with the slowest speed of the kymograph compatible with a clear demonstration of the points in the tracings which it is desired to stress. Large areas of black background should not be included. Needlessly long tracings lose much of their detail when reduced in size for reproduction. The original size of the tracing should be indicated by inserting a cm. scale.

(7) *Lettering.* Kymograph records are often sent in with letters and numbers on the tracing. Unless special instructions are given, these are not redrawn by the press. If they are intended for reproduction they should be large enough to stand the reduction which must take place when the block is made. If not, they should be blacked out.

(8) *Half-tone reproductions.* In the process of reproduction a photograph loses a certain amount of definition and contrast. Thus a string galvanometer record in which the string shadow is visible but faint may make a disappointing illustration even if it is printed on "art" paper as a special plate. Good photographic technique in the first instance is the best remedy, but photographs with little contrast can be strengthened by the use of an intensifier, or by copying them on to "process" plates. The Editors and the Press cannot undertake to "touch up" photographs or kymograph tracings, and if this is done extensively it should be stated in the legend to the figure.

(9) *Plates.* Half-tone illustrations are usually printed in the body of the text on a slightly glazed paper. If they have too much detail or too little contrast they can be printed as a special plate on highly glazed paper. As this involves additional expense the illustrations must be carefully chosen and arranged so that space is not wasted.

10. References

References should be restricted to those which will help a reader to understand the paper. No attempt should be made to give a bibliography of the subject under discussion.

It is of the utmost importance that references should invariably be verified and given accurately. If quotation at second hand is unavoidable, in any case the exact source of the information quoted should be given, *e.g.* Smith, A. B. (1920), quoted from Brown, C. D. (1922); or Smith, A. B. (1920), quoted from *Physiol. Abst.* 1921, **6**, 729.

References should all be collected together at the end of the paper and should be arranged there in alphabetical order of authors' names. Each reference gives:

(1) Author's name followed by initials.

(2) Year of publication in brackets.

(3) Journal in which the paper referred to appeared. The titles of journals should be abbreviated in accordance with the system of the World List of Scientific Periodicals and should be printed in italics (instruction to printer: single underline in MS.). The World List abbreviations of the titles most commonly quoted in physiological papers are given in Appendix 2.

(4) Volume number. For this arabic numerals are used in clarendon type. The prefix "vol." is not to be inserted.

(5) Number of first page of article in arabic numerals in ordinary type, or, if the reference is to a book, the actual page. The prefix "p." is not to be inserted.

If references to several papers published by a certain author in any one year have to be given, the letters *a*, *b*, *c*, etc., are placed after the year of publication and within the bracket. If two or more references to the same journal follow one another on consecutive lines, the name of the journal is not repeated but *ibid.* is used. With this exception the use of *ibid.* is to be avoided. If the reference is to a book the title should be followed by the place of publication and (in brackets) the publisher's name. These must both be written in full and printed in roman type. The following examples may make the method clearer:

Hermann, L. (1878). *Pflügers Arch.* **18**, 209.
Mines, G. R. (1912*a*). *Kolloidchem. Beitr.* **3**, 191.
Mines, G. R. (1912*b*). *J. Physiol.* **44**, 138.
Taylor, N. B. (1931). *Ibid.* **71**, 156.

When it is needful to cite particular parts, fascicles, numbers, etc., if the article referred to is paginated in roman numerals, or in other cases where strict adherence to the system here given might cause confusion, ordinary abbreviations, *e.g.* vol., p., fasc. (or f.), No., etc., may be used. References to *Proceedings of the Physiological Society* are given thus: *J. Physiol.* **69**, 13*P*. The references are cited in the text by the author's name and date only. Thus: Mines [1912*b*].

The expression "*loc. cit.*" must never be used.

11. Abbreviations

It is desirable in the interests of economy and brevity that abbreviations should be used freely. But on the other hand it is essential that any abbreviations used must be readily understood by readers and be free from ambiguity.

The following abbreviations and signs are both allowable and desirable for units of the metric system:

kilometre, km.	Angstrom unit (10^{-7} mm. or 100 $\mu\mu$), A.U.	hectolitre, hl.
metre, m.	kilogram, kg., *not* kilo.	decalitre, dl.
centimetre, cm.	gram, g. *not* gm.	litre, l.
millimetre, mm.	centigram, cg.	millilitre, ml.
micron (0·001 mm.), μ.	milligram, mg.	cubic centimetre, c.c.
millimicron (10^{-6} mm.), mμ.	0·001 mg., γ.	cubic millimetre, c.mm.
millionth micron (10^{-9} mm.) $\mu\mu$.	kilolitre, kl.	sigma (0·001 second), σ.
microfarad, μF.	minute, min.	kilogram-metre, kg.m.
		second, sec.

The abbreviation "g.p.c." or "mg.p.c." is not allowable. The correct form is "g./100 g." or "g./100 c.c." or "mg./100 g." or "mg./100 c.c."

The same abbreviations are used for both singular and plural, *e.g.* 0·5 cm., 1·5 cm., 10 cm., *not* 0·5 cms., 1·5 cms., 10 cms. For measures of area the letters "sq." should be prefixed to the abbreviation for the corresponding linear unit, The sign ^{2} is not to be used. For cubic measure the letter "c" is prefixed similarly. An exception to this rule is c.c. not c.cm. to conform to established usage. Care should be taken, when any possible doubt can arise, to write "gram" and "grain" in full. Otherwise the abbreviation gr. may be used for "grain." Measures of temperature must always indicate the thermometric scale used, and for this purpose the abbreviations C. or F. are affixed to the numeral, *e.g.* 57° C., 98·4° F.

The sign % must not be used to indicate percentages. This should always be written p.c.

In general, however, except in cases of established custom, concentrations should be expressed as multiples or fractions of normality, *e.g.* it is better to write "the concentration of hydrochloric acid in the gastric juice was 0·055 N" rather than 0·2 p.c.

The forms *c*H and *p*H are to be used.

An abbreviation, but not a sign, is always followed by a full stop.

12. Mathematical Notation and Numerals

With regard to mathematical notation the rules laid down in *Proceedings of the Royal Society*, A, 1909, **82**, 14, should be followed.

A numeral should never be used to start a sentence, but numerals may be used in the text as abbreviations of words.

The decimal point must always be preceded by a figure, if necessary 0 being prefixed, *e.g.* 0·1 must be written, *never* ·1.

In a column of numbers containing decimal fractions the decimal points should always be arranged under one another in the same vertical line and the same number of figures should be given in each case, 0's being used if necessary:

e.g.

15·623		15·623
1·000	not	1
0·796		·796
112·430		112·43

Needless repetition of 0's in columns should, when possible, be avoided *e.g.* by writing powers of 10 (10^3, 10^{-7} etc.) at the head of the column. The same notation may often be used in the text, but need not be adhered to slavishly.

Fractions and ratios must be written 1/100 not $\frac{1}{100}$.

13. Chemical Nomenclature

The rules of the Chemical Society should be followed. In particular the following should be borne in mind.

Chemical symbols do not have a full stop placed after them unless they come at the end of a sentence.

(1) A hydrate is a compound containing water of crystallization or combination. A compound of a metallic radicle with the hydroxyl group is a hydroxide, *e.g.* sodium hydroxide, NaOH.

(2) Such terms as acid sodium phosphate should be avoided. Salts of acids containing more than one replaceable hydrogen atom should be described by naming the actual cations they contain, *e.g.* sodium dihydrogen phosphate and disodium hydrogen phosphate.

(3) Substances containing basic groups have names terminating in *ine* not *in*: *e.g.* aniline, choline, creatine, strychnine, etc.

The termination *in* is restricted to certain neutral compounds (carbohydrates, glycerides, glucosides, proteins): *e.g.* dextrin, pepsin, albumin, etc.

(4) Protein is used, *never* proteid. Most of the amino acids follow rule (3): *e.g.* leucine, glycine, thyroxine, etc. Tryptophan does not have a final *e*, since *ane* is the termination reserved for saturated hydrocarbons, *e.g.* methane.

(5) All alcohols (hydroxyl derivatives of hydrocarbons) should have names ending in *ol* not *ite* or *ine*: *e.g.* glycerol (when used as a chemical term), mannitol, etc.

Compounds which are not alcohols, but which are commonly given names ending in *ol*, should be spelt with a terminal *e*: *e.g.* indole, iminazole, pyrrole, etc.

(6) Lipoid (or better lipoidal) is an adjective. Fatty substances containing nitrogen or nitrogen and phosphorus (lecithine, kephaline, etc.) are lipines.

In any cases of doubt the collective Index of the *Transactions and Abstracts of the Chemical Society*, 1913–22, Part II, Index of Subjects, should be followed.

Trade names cause some difficulty. It is perhaps best to put them in inverted commas only where first used in a paper and to state that thereafter the name refers to a particular preparation.

14. Summary

All papers should conclude with a summary of their contents. This summary should be arranged in short, numbered paragraphs, and should not exceed in length 5 p.c. of the paper.

15. Decimal System

The decimal system of the *Institut International de Documentation* has been adopted by the *Journal of Physiology* in common with other journals, with the object of providing a subject classification of papers and reprints. It would be of great help to the Editors if authors would insert the appropriate decimal number at the beginning of their MS.

The tables of decimal numbers may be obtained from a bookseller.

APPENDICES I AND II

APPENDIX I

PROOF CORRECTION

[Proof]

1. s.c. — Results
2. indent — Most of the experiments in the present
3. 9/ — investigation were carried out with
4. ,/ — cocaine, and the results to be described
5. below/ — ~~blow~~ were obtained with this unless
6. #/ — special mention is made.
7. Ital/ — Magnitude of action potential.
8. 9/ — (1) Conduction along the distal ~~non~~ non-
9. Rom/ — narcotised segment. In view of the
10. tr/ — finding of *Woronzow* that the size
11. tr/ — of electric response suffers decrement
12. tr/ — along the distal region most beyond the
13. sce/ — depressed area, it is interesting, first
14. λ the — of all, to ~~find~~ whether the same
15. /run on — would occur under present experimental
16. DD/caps — conditions.) For this purpose ~~all~~ leads were first
17. L/ — employed. Records L of L propagated
18. stet/ — disturbances were taken ~~both~~ before and
19. x/ — at varying intervals during narcosis and
20. q/ — recovery. With ND leads diphasic re-
21. l.c/ — cords were obtained in which the POTENTIAL
22. wf/ — variations appearing in the narcotised
23. -/ — and non narcotised distal regions are
24. =/ — simultaneously represented in the two
25. ~~~ — phases of each response, the first phase
26. — — being derived from the former and the
27. ⊙/ — second from the latter.

Explanation of marks.

1. To change to small capitals.
2. When a word is too far to the left.
3. When a letter is inverted.
4. Substitution of comma for another point or letter. Similarly for colon and semi-colon.
5. When letters in a word are misplaced.
6. Insertion of a space.
7. When italics should be used.
8. When a letter or word is to be omitted.
9. When a word is to be changed to roman type.
10, 11, 12. Methods of marking transposition. When several words are badly mixed up they may be numbered, the usual mark being placed in the margin.
13. Substitution of one word for another.

[The same corrected]

RESULTS

Most of the experiments in the present investigation were carried out with cocaine, and the results to be described below were obtained with this unless special mention is made.

Magnitude of action potential.

(1) *Conduction along the distal non-narcotised segment.* In view of the finding of Woronzow that the size of the electric response suffers most decrement along the distal region beyond the depressed area, it is interesting, first of all, to see whether the same would occur under the present experimental conditions. For this purpose DD leads were first employed. Records of propagated disturbances were taken both before and at varying intervals during narcosis and recovery.

With ND leads diphasic records were obtained in which the potential variations appearing in the narcotised and non-narcotised distal regions are simultaneously represented in the two phases of each response, the first phase being derived from the former and the second from the latter.

Explanation of marks (cont.).

14. When a letter or word is to be inserted.
15. When a paragraph occurs wrongly.
16. Substitution of capitals for small letters.
17. To reduce spacing.
18. When words crossed out are to remain.
19. When letters appear imperfectly.
20. Mark for a paragraph.
21. To change capitals to small letters.
22. When a letter from a wrong fount has been used.
23. To insert a hyphen.
24. When letters or words are out of line.
25. When Clarendon type should be used.
26. When letters are to be brought close together.
27. Insertion of full stop when omitted or replaced by another point.

APPENDIX II

List of Periodicals and their Abbreviations

Periodical	Abbreviation
Acta Medica Scandinavica	Acta med. scand.
Acta Ophthalmologica	Acta ophthal., Copenhagen
Acta Scholae medicinalis Universitatis Imperialis in Kioto	Acta Sch. med. Univ. Kioto
American Journal of Anatomy	Amer. J. Anat.
American Journal of Diseases of Children	Amer. J. Dis. Child.
American Journal of Hygiene	Amer. J. Hyg.
American Journal of the Medical Sciences	Amer. J. med. Sci.
American Journal of Pharmacy	Amer. J. Pharm.
American Journal of Physiology	Amer. J. Physiol.
American Journal of Psychology	Amer. J. Psychol.
American Journal of Tropical Diseases	Amer. J. trop. Dis.
Anatomical Record	Anat. Rec.
Anatomischer Anzeiger	Anat. Anz.
Annales de Chimie	Ann. Chim.
Annales de Chimie et de Physique	Ann. Chim. Phys.
Annales de l'Institut Pasteur	Ann. Inst. Pasteur, Paris
Annales de Médecine	Ann. Méd.
Annales de Physiologie et de Physico-Chimie biologique	Ann. Physiol., Paris
Annales de Physique	Ann. Phys., Paris
Annales de la Société Royale des sciences médicales et naturelles de Bruxelles	Ann. Soc. Sci. méd. nat. Brux.
Annali d' Igiene	Ann. Igi.
Annals of Applied Biology	Ann. appl. Biol.
Arbeitsphysiologie	Arbeitsphysiol.
Archiv für Anatomie und Pharmakologie	Arch. Anat. Pharmak.
Archiv für Anatomie und Physiologie	Arch. Anat. Physiol., Lpz.
Archiv für Augenheilkunde	Arch. Augenheilk.
Archiv für Entwicklungsmechanik der Organismen	Arch. EntwMech. Org.
Archiv für experimentelle Pathologie und Pharmakologie	Arch. exp. Path. Pharmak.
Archiv für die gesammte Physiologie	Pflügers Arch.
Archiv für Hygiene	Arch. Hyg., Berl.
Archiv für mikroskopische Anatomie	Arch. mikr. Anat.
Archiv für Ophthalmologie	Graefes Arch. Ophthalm.
Archiv für pathologische Anatomie	Virchows Arch. path. Anat.
Archiv für Verdauungskrankheiten	Arch. VerdauKrankh.
Archives de Biologie	Arch. Biol., Paris
Archives of Internal Medicine	Arch. intern. Med.
Archives internationales de Pharmacodynamie et de Thérapie	Arch. int. Pharmacodyn.
Archives internationales de Physiologie	Arch. int. Physiol.
Archives italiennes de Biologie	Arch. ital. Biol.
Archives de médecine expérimentale et d'anatomie pathologique	Arch. Méd. exp.
Archives néerlandaises de Physiologie	Arch. néerland. Physiol.
Archives of Neurology and Psychiatry, Chicago	Arch. Neurol. Psychiat., Chicago
Archives of Neurology and Psychiatry, London	Arch. Neurol. Psychiat., Lond.
Archives d'Ophtalmologie	Arch. Ophtal., Paris
Archives of Ophthalmology	Arch. Ophthal., N.Y.
Archives de Physiologie normale et pathologique	Arch. Physiol. norm. path.
Archives des Sciences biologiques	Arch. Sci. biol.
Archivio di Fisiologia	Arch. Fisiol.
Archivio per le Scienze mediche	Arch. Sci. med., Torino

Periodical	Abbreviation
Atti della R. Accademia dei Lincei (Memorie)	Mem. Accad. Lincei
Atti della R. Accademia dei Lincei (Rendiconti)	R.C. Accad. Lincei
Atti della R. Accademia delle Scienze di Torino	Atti Accad. Torino
Atti della Società lombarda di Scienze mediche e biologiche	Atti Soc. lombarda Sci. med. biol.
Atti del R. Istituto veneto di scienze, lettere ed arti	Atti Ist. veneto
Australian Journal of Experimental Biology and Medical Science	Aust. J. exp. Biol.
Beiträge zur chemischen Physiologie und Pathologie	Beitr. chem. Physiol.
Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie	Beitr. path. Anat.
Berichte der deutschen chemischen Gesellschaft	Ber. dtsch. chem. Ges.
Berichte über die gesammte Physiologie und experimentelle Pharmakologie	Ber. ges. Physiol.
Berliner klinische Wochenschrift	Berl. klin. Wschr.
Bibliothek för Laeger	Bibl. Laeger
Biochemical Journal	Biochem. J.
Biochemische Zeitschrift	Biochem. Z.
Biological Bulletin of the Marine Biological Laboratory, Wood's Hole	Biol. Bull. Wood's Hole
Biological Reviews	Biol. Rev.
Biologisches Zentralblatt	Biol. Zbl.
Bollettino della Società italiano di biologia sperimentale	Boll. Soc. ital. Biol. sper.
Brain	Brain
British Journal of Children's Diseases	Brit. J. Child. Dis.
British Journal of Experimental Biology	Brit. J. exp. Biol.
British Journal of Experimental Pathology	Brit. J. exp. Path.
British Journal of Ophthalmology	Brit. J. Ophthal.
British Journal of Psychology	Brit. J. Psychol.
British Medical Journal	Brit. med. J.
Bulletin de l'Académie de Médecine de Belgique	Bull. Acad. Méd. Belg.
Bulletin de l'Académie de Médecine de Paris	Bull. Acad. Méd. Paris
Bulletin et Mémoires de la Société des hôpitaux de Paris	Bull. Soc. méd. Hôp. Paris
Bulletin de la Société de Chimie biologique	Bull. Soc. Chim. biol., Paris
Carnegie Institution of Washington Publications	Publ. Carnegie Instn.
Chemical Abstracts	Chem. Abstr.
Chemische Zeitschrift	Chem. Z.
Chinese Journal of Physiology	Chinese J. Physiol.
Chinese Journal of Physiology, Report Series, metabolism	Chinese J. Physiol. Rep. Metabol.
Comptes rendus hebdomadaires des séances de l'Acad. des Sciences	C. R. Acad. Sci. Paris
Comptes rendus Laborstoire Carlsberg	C. R. Lab. Carlsberg
Comptes rendus de la Société de Biologie	C. R. Soc. Biol., Paris
Deutsche medizinische Wochenschrift	Dtsch. med. Wschr.
Deutsche Zeitschrift für Nervenheilkunde	Dtsch. Z. Nervenheilk.
Endocrinology	Endocrinology
Endokrinologie	Endokrinologie
Ergebnisse der Physiologie	Ergebn. Physiol.
Fermentforschung	Fermentforsch.
Folia Hæmatologica	Folia hæmat. Lpz.
Graefes Archiv	Graefes Arch. Ophthalm.
Guy's Hospital Reports	Guy's Hosp. Rep.
Hæmatologica	Hæmatologica
Handbuch der biochemischen Arbeitsmethoden	Handb. biochem. ArbMeth.

Periodical	Abbreviation
Handbuch der biologischen Arbeitsmethoden	Hand. Biol. ArbMeth.
Harvey Lectures	Harvey Lect.
Heart	Heart
Hoppe-Seylers Zeitschrift	Z. physiol. Chem.
Indian Journal of Medical Research	Ind. J. med. Res.
Japanese Journal of Medical Sciences	Jap. J. med. Sci.
Part 2. Biochemistry	Biochem.
Part 3. Biophysics	Biophys.
Johns Hopkins Hospital Bulletin	Johns Hopk. Hosp. Bull.
Johns Hopkins Hospital Reports	Johns Hopk. Hosp. Rep.
Journal of the American Chemical Society	J. Amer. chem. Soc.
Journal of the American Medical Association	J. Amer. med. Ass.
Journal de l'Anatomie et de la Physiologie	J. Anat., Paris
Journal of Anatomy	J. Anat., Lond.
Journal of Bacteriology	J. Bact., Baltimore
Journal of Biochemistry	J. Biochem.
Journal of Biological Chemistry	J. biol. Chem.
Journal of Biophysics	J. Biophys., Tokyo
Journal of the Canadian Medical Association	J. Canad. med. Ass.
Journal of Cellular and Comparative Physiology	J. cell. comp. Physiol.
Journal of the Chemical Society	J. chem. Soc., Lond.
Journal of Clinical Investigation	J. clin. Invest.
Journal of Comparative Neurology	J. comp. Neurol.
Journal of Experimental Biology	J. exp. Biol.
Journal of Experimental Medicine	J. exp. Med.
Journal of Experimental Psychology	J. exp. Psychol.
Journal of Experimental Zoology	J. exp. Zool.
Journal of General Physiology	J. gen. Physiol.
Journal of Genetics	J. Genet.
Journal of Hygiene	J. Hyg., Camb.
Journal of Infectious Diseases	J. infect. Dis.
Journal of Laboratory and Clinical Medicine	J. Lab. clin. Med.
Journal of the Marine Biological Association	J. mar. biol. Ass. U.K.
Journal of Medical Research	J. med. Res.
Journal of Metabolic Research	J. metabol. Res.
Journal of the Optical Society of America	J. opt. Soc. Amer.
Journal of Pathology and Bacteriology	J. Path. Bact., Lond.
Journal de Pharmacie et de Chimie	J. Pharm. Chim., Paris
Journal of Pharmacology and Experimental Therapeutics	J. Pharmacol., Baltimore
Journal de Physiologie et de Pathologie générale	J. Physiol. Path. gén.
Journal of Physiology	J. Physiol.
Journal de Psychologie normale et pathologique	J. Psychol. norm. path.
Journal of the Royal Microscopical Society	J. R. micr. Soc.
Journal of Scientific Instruments	J. sci. Instr.
Klinische Wochenschrift	Klin. Wschr.
Kolloid-Zeitschrift	Kolloid-Z.
Lancet	Lancet
Liebig's Annalen der Chemie	Liebigs Ann. Chem.
Medical Journal of Australia	Med. J. Aust.
Medical Journal of South Africa	Med. J. S. Afr.
Medical Science, Abstracts and Reviews	Med. Sci.
Monatsschrift für Kinderheilkunde	Mschr. Kinderheilk.
Monitore zoologico italiano	Monit. zool. ital.
Morgagni	Morgagni
Münchener medizinische Wochenschrift	Münch. med. Wschr.
Nature	Nature
Naturwissenschaften	Naturwissenschaften
Nederlandsch Tijdschrift voor Geneeskunde	Ned. T. Geneesk.

Periodical	Abbreviation
Oppenheimer's Handbuch der Biochemie	Oppenheimer's Handb. Biochem.
Pflügers Archiv	Pflügers Arch.
Philosophical Magazine	Philos. Mag.
Philosophical Transactions	Philos. Trans.
Physikalische Zeitschrift	Phys. Z.
Physiological Abstracts	Physiol. Abstr.
Physiological Reviews	Physiol. Rev.
Policlinico	Policlinico
Presse médicale	Presse méd.
Proceedings of the National Academy of Sciences, Washington	Proc. Nat. Acad. Sci. Wash.
Proceedings of the Physiological Society	Proc. Physiol. Soc.
Proceedings of the Royal Academy of Sciences, Amsterdam	Proc. Acad. Sci. Amst.
Proceedings of the Royal Dublin Society	Sci. Proc. R. Dublin Soc.
Proceedings of the Royal Society	Proc. Roy. Soc.
Proceedings of the Royal Society of Canada	Proc. Roy. Soc. Can.
Proceedings of the Royal Society of Edinburgh	Proc. Roy. Soc. Edinb.
Proceedings of the Royal Society of Medicine	Proc. R. Soc. Med.
Proceedings of the Society of Experimental Biology and Medicine	Proc. Soc. exp. Biol., N.Y.
Pubblicazioni della Stazione zoologica di Napoli	Pubbl. Staz. zool. Napoli
Quarterly Journal of Experimental Physiology	Quart. J. exp. Physiol.
Quarterly Journal of Medicine	Quart. J. Med.
Quarterly Journal of Microscopical Science	Quart. J. micr. Sci.
Rendiconti della R. Accademia dei Lincei	R. C. Accad. Lincei
Reports of British Association	Rep. Brit. Ass.
Riforma Medica	Riforma med.
Rivista di biologia	Riv. Biol.
Science	Science
Science Abstracts	Sci. Abstr.
Sitzungsbericht der Akademie der Wissenschaften in Wien	S.-B. Akad. Wiss. Wien
Skandinavisches Archiv für Physiologie	Skand. Arch. Physiol.
South African Journal of Science	S. Afr. J. Sci.
Special Report Series Medical Research Council, London	Sp. Rep. Ser. Med. Res. Coun.
Sperimentale. Archivio di biologia normale e patologica	Sperimentale
Stanford University Publications—University Series Biological Sciences Medical Sciences	Stanford Univ. Publ. biol. Sci. med. Sci.
Tohoku Journal of Experimental Medicine	Tohoku J. exp. Med.
Transactions of the Faraday Society	Trans. Faraday Soc.
Transactions of the Optical Society	Trans. Opt. Soc. Lond.
Transactions of the Royal Society of Canada	Trans. Roy. Soc. Can.
Transactions of the Royal Society, Edinburgh	Trans. Roy. Soc. Edinb.
Transactions of the Royal Society, South Africa	Trans. Roy. Soc. S. Afr.
Travaux du laboratoire des recherches biologiques de l'université de Madrid	Trav. Lab. Rech. biol. Madr.
Ugeskrift för Laeger	Ugeskr. Laeg.
University of California Publications	Univ. Calif. Publ.
Verhandeligen der (K) Akademie van Wetenschappen	Verh. Akad. Wet. Amst.
Verhandlungen der Gesellschaft Deutscher Naturforscher und Ärzte	Verh. Ges. dtsch. Naturf. Ärzte
Wiener klinische Wochenschrift	Wien. klin. Wschr.
Wiener medizinische Wochenschrift	Wien. med. Wschr.

Periodical	Abbreviation
Zeitschrift für allgemeine Physiologie	Z. allg. Physiol.
Zeitschrift für Augenheilkunde	Z. Augenheilk.
Zeitschrift für Biologie	Z. Biol.
Zeitschrift für experimentelle Pathologie und Therapie	Z. exp. Path.
Zeitschrift für die gesammte Anatomie	Z. ges. Anat.
Zeitschrift für die gesammte experimentelle Medizin	Z. ges. exp. Med.
Zeitschrift für Hygiene und Infektionskrankheiten	Z. Hyg. Infektkr.
Zeitschrift für Immunitätsforschung	Z. Immunitätsforsch.
Zeitschrift für Kinderheilkunde	Z. Kinderheilk.
Zeitschrift für klinische Medizin	Z. klin. Med.
Zeitschrift für Kreislaufforschung	Z. Kreislaufforsch.
Zeitschrift für die medizinische Wissenschaften	Z. med. Wiss.
Zeitschrift für physikalische Chemie	Z. phys. Chem.
Zeitschrift für physiologische Chemie	Z. physiol. Chem.
Zeitschrift für Psychologie und Physiologie der Sinnesorgane	Z. Psychol. Physiol. Sinnesorg.
Zeitschrift für Sinnesphysiologie	Z. Sinnesphysiol.
Zeitschrift für vergleichende Physiologie	Z. vergl. Physiol.
Zeitschrift für wissenschaftliche Zoologie	Z. wiss. Zool.
Zeitschrift für Zellforschung und mikroskopische Anatomie	Z. Zellforsch.
Zentralblatt für Biochemie und Biophysik	Zbl. Biochem.
Zentralblatt für die medizinische Wissenschaften	Zbl. med. Wiss.
Zentralblatt für Physiologie	Zbl. Physiol.

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